Chemical Information Review Document

for

Hydroxyurea
[CAS No. 127-07-1]

Supporting Nomination for Toxicological Evaluation by the National Toxicology Program

November 2011

National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S Department of Health and Human Services
Research Triangle Park, NC
http://ntp.niehs.nih.gov/

Abstract

Hydroxyurea (HU) is currently the only approved disease-modifying therapy for sickle cell disease. It has also been approved for treatment of select cancers (e.g., melanoma; resistant chronic myelocytic leukemia; and recurrent, metastatic, or inoperable ovarian carcinoma) and approved for concomitant use with irradiation therapy for treatment of primary squamous cell carcinomas of the head and neck, excluding the lip. High performance liquid chromatography and gas chromatography/mass spectrometry have been widely used to detect HU. HYDREA® and DROXIA®, produced by Bristol Myers Squibb Company, contain HU. In addition to its approved uses, HU has been used for the treatment of other disorders, including essential thrombocythaemia, spinal muscular atrophy, intracranial meningiomas, and desmoid tumor. Several clinical trials have evaluated or are evaluating the efficacy of HU for treatment of malignant glioma, spinal muscle atrophy, human immunodeficiency virus, chronic myelogenous leukemia, locally advanced nasopharyngeal carcinoma, advanced mouth cancer, and advanced stomach cancer. Several studies are or were evaluating the effects of HU in children treated for sickle cell disease. The Best Pharmaceuticals for Children Act requires identification and prioritization of approved drugs for which studies in children are needed. Under this act, HU was initially listed in 2006. HU levels in male Sprague-Dawley rats were lower in the brain when compared to the plasma after injection or injection and infusion of radiolabeled HU. Calculated distribution and elimination half-lives were 1.7 and 171 minutes, respectively. The brain-to-plasma ratio at 60 minutes post-administration ranged from 0.13 to 0.18. HU was not metabolized by human or rat liver microsomes, in the presence or absence of nicotinamide adenine dinucleotide phosphate. Additionally, HU was not a substrate for P-glycoprotein *in vitro*. Comparatively, *in vitro* incubation of HU with rat liver homogenates increased nitrate and nitrite formation; NADPH addition decreased nitrate and nitrite formation. Oral LD_{50} values in mice and rats were 7330 and 5760 mg/kg, respectively. Intraperitoneal and intravenous LD_{50} values in both species were >2300 mg/kg. Cardiovascular and hematological adverse effects were noted in some laboratory animals after subchronic or short-term exposure to doses that exceeded clinical levels. Bone marrow hypoplasia, pulmonary congestion, and mottling of lungs were also noted in rats in short-term studies. Calcium channel blockers enhanced cell growth inhibition induced by HU in a human intraosseous malignant meningioma cell line. In HL-60 and T24 cells, HU blocked the azacitidine and decitabine-mediated inhibition of DNA methylation in a dose-dependent manner. Studies indicate that HU is cytotoxic to a variety of cells (e.g., immortalized human neural progenitor [ReNcell CX] cells). HU significantly increased the apoptotic index in HUVEC cells. Comparatively, addition of laminar shear stress inhibited HU-induced apoptosis. HU produces adverse reproductive and teratological effects. Recent studies in rats showed that HU decreased Bcl-2 expression and increased Fas and Fast expression in testes and the formation of DNA lesions in testicular cells. HU also altered testis weight and dimensions in transgenic sickle cell mice. It significantly inhibited proliferation and differentiation of midbrain cells harvested from 13-day-old rat embryos. Fetuses exposed to HU *in utero* showed numerous skeletal variations and malformations. The no observed effect level (NOEL) for observed variations was <250 mg/kg, while the NOEL for observed malformations was 250 mg/kg. *Spirulina maxima* and dimethyl sulfoxide antagonized the teratogenic and developmental effects in mice and mouse embryos, respectively, produced by HU. Carcinogenicity studies are conflicting; one study reported that HU increased the incidence of mammary tumors in rats, while another reported that pulmonary tumor formation was not affected by HU administration. HU produced mutagenic effects in bacteria, fungi, protozoa, and mammalian cells. Clastogenic effects have been noted *in vitro* in hamster cells and human lymphoblasts and *in vivo* in rodents. HU also produces epigenetic effects. It increased DNA hypermethylation in HTB-54 lung human epidermoid carcinoma cells after 24- and 28-hour incubation periods. Microarray studies show that HU upregulated pro-inflammatory cytokine expression 2- to 30-fold. In a GeneGo quantitative-structure activity relationship analysis, HU was predicted to be carcinogenic and heptatotoxic. Based on the predicted targets, HU is proposed to affect one-carbon metabolic process and deoxyribonucleotide biosynthetic and metabolic processes.

Executive Summary

Basis for Nomination

Hydroxyurea (HU) was nominated by the National Institute of Environmental Health Sciences for toxicological testing based on long-term safety concern when used as therapy for sickle cell anemia. HU is approved for treatment of sickle cell disease and certain cancers in adults. However, it is being used increasingly in young children and infants for treatment of sickle cell disease. There is limited information on the potential long-term consequences of HU use in infants, children, and adults. The need for multi-generation experimental animal studies were identified as a critical data need by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (CERHR; renamed the Office of Health Assessment and Translation) Expert Panel. These studies are proposed to assess the long-term effects of prenatal and postnatal exposures on postnatal development.

Nontoxicological Data

HU was initially used 25 years ago in a clinical study of adults with sickle cell disease. It is currently the only approved disease-modifying therapy for sickle cell disease. It has also been approved for treatment of select cancers (e.g., melanoma; resistant chronic myelocytic leukemia; and recurrent, metastatic, or inoperable ovarian carcinoma). It was also approved for concomitant use with irradiation therapy for treatment of primary squamous cell carcinomas of the head and neck, excluding the lip. High performance liquid chromatography and gas chromatography/mass spectrometry have been widely used to detect HU. Electrochemical or ultraviolet detection methods have been used to detect underivatized HU. Detection of a derivatized form of HU has also been described. Bristol Myers Squibb Company produces two brand name prescription drugs whose active ingredient is HU, HYDREA® and DROXIA®. Barr Laboratories Inc. and Par Pharmaceutical Inc produce generic equivalents. There are numerous U.S. and global suppliers and producers of HU. It can be synthesized by reacting hydroxylamine hydrochloride with potassium cyanate in aqueous solution or calcium cyanate with hydroxylamine nitrate in absolute ethanol. An alternate synthetic pathway was described in 1955 where a quaternary ammonium ion exchange resin was reacted with a sodium cyanate to convert the resin to the cyanate form. The resin is then reacted with hydroxylamine hydrochloride to produce HU. In addition to its approved uses, HU has been used for the treatment of essential thrombocythaemia, spinal muscular atrophy, and intracranial meningiomas. Several clinical trials have also evaluated or are evaluating the efficacy of HU for treatment of malignant glioma, spinal muscle atrophy, human immunodeficiency virus, chronic myelogenous leukemia, locally advanced nasopharyngeal carcinoma, advanced mouth cancer, and advanced stomach cancer. Several studies are or were evaluating the effects of HU in children with sickle cell disease. The Best Pharmaceuticals for Children Act requires identification and prioritization of approved drugs for which studies in children are needed. Under this act, HU was initially listed in 2006.

Human Data

Efficacy

Although HU is the only approved disease-modifying therapy for sickle cell disease in adults, it is being used for the treatment of sickle cell disease also in children as well as of other disorders (e.g., polycythemia vera). In patients with sickle cell disease, HU increased hemoglobin and fetal hemoglobin levels and average blood cell volume, while decreasing events/parameters such as the rate of vaso-occlusive crisis, acute chest syndrome, transfusions, polynuclear neutrophils, and platelets. Decreases in the number of transfusions were also reported in patients with thalassemia. More recently, HU was found to be an alternative therapy to treat secondary polycythemia caused by incurable cyantic congenital heart disease (CCHD). It was also effective in the treatment of secondary erythrocytosis in CCHD. HU improved symptoms of hyperviscosity (e.g., fatigue, dyspnea, and recurrent transient ischemic attack) in patients while decreasing red blood cell count and increasing both mean corpuscular hemoglobin and volume. HU chemotherapy was also recently used to treat a 12-year-old boy for 18 months to prevent the

occurrence of desmoid tumor. A recent case report described the use of HU to treat chronic immune thrombocytopenic purpura in a woman with sickle cell disease.

Adverse Effects

HU treatment is not without its side effects. It induced cutaneous lesions typical of amyopatic dermatomyositis, usually months of years after the start of treatment. In most patients, improvement of clinical manifestations occurred following discontinuation of HU. Of 27 patients treated with HU, 89% presented with cutaneous or mucous side effects (e.g., cutaneous xerosis). Side effects were associated with a cumulative dose of 2 kg. In a separate study, 210 patients with a variety of diseases (essential thrombocythemia, chronic myelogenous leukemia, polycythemia vera, and chronic myeloproliferative syndromes) were monitored for the development of skin lesions. Of the examined patients, 30 developed skin-related side effects: melanonychia, acral ulcers, dermopathy, multiple cutaneous carcinomas, and diffuse melanosis. Out of 124 patients with essential thrombocythemia, polycythemia vera, and myelofibrosis, five women over the age of 75 had developed leg ulcers. Although effective for patients with myeloproliferative disorders, effects such as macrocytic anemia, fever, myelodysplasia, and leg ulcers have also been reported. In most cases, the effects were minor compared to symptoms of the disorder and were reversed upon the cessation of HU therapy.

In children with sickle cell disease with avascular necrosis of the femoral head (AVN), the presence of AVN was significantly associated with HU use. HU was implicated as the causal drug for drug-induced hypersensitivity syndrome in one individual. Two case reports included multiple cutaneous neoplasms, leg ulcers, multiple hypertrophic actinic keratoses, and squamous cell carcinoma. Of 136 patients with essential thromocythemia and undergoing HU treatment, six experienced hemorrhagic events and 26 experienced thrombotic events. In a retrospective study, 11.2% of patients treated with HU developed a second malignancy after diagnosis with essential thromocythemia; no association between treatment and an increased risk of the development of a second hematological malignancy was established.

Several case reports have also described adverse effects associated with HU treatment. For example, an elderly woman with polycythemia vera undergoing HU treatment developed pulmonary thrombovasculities and desquamation alveolitis and eventually died. In another case report involving an elderly woman, exposure to HU for 13 years was associated with development of cutaneous squamous dysplasia; evaluations of lesions indicated development of Bowenoid AK and Bowen disease. In another case report,. long-term HU therapy led to formation of cutaneous lesions which then progressed to basal cell carcinoma and poorly differentiated sarcomatoid squamous cell carcinoma. Three recent case reports have described the development of melanonychia associated with HU treatment for varying lengths of time. Development of porokeratosis, which is manifested clinically as annular plaques with a peripheral keratotic rim, was described in two men with polycythemia rubra vera that were being treated with HU. HU-induced hyperkalemia was proposed in an 81-year old woman diagnosed with polycythemia vera; length of HU-treatment was not provided.

Compared to these studies, a lack of effect was observed in a child that received an accidental overdose of HU. An 11-month-old infant with sickle cell anemia ingested 9800 mg (612 mg/kg) HU. Serum level of HU 4 hours after ingestion was ~7756 μM. While transient hematological effects were noted (e.g., reduction in leukocyte counts), no liver or renal effects were observed. No other effects were reported, and complete recovery of all effects was noted by 7 days after exposure.

Reproductive and Teratological Studies

Overall, studies conducted in individuals taking HU showed no adverse effects in parent reproductive parameters or in offspring. In two pregnant women who had been taking HU for treatment of sickle-β thalassemia (but discontinued upon discovering they were pregnant), no abnormal congenital malformations were observed in their babies. More than 30 pregnant women with sickle cell disease or

with different myeloproliferative disorders exposed to HU in the first, second, or third trimester delivered healthy babies. Additional studies reported no teratogenic or mutagenic effects in newborns of 19 women who had taken HU. HU was even used in place of imatinib to treat a 36-year-old pregnant patient with chronic myeloid leukemia; a healthy baby was delivered. In a 17-year follow-up of surviving patients enrolled in the original Multicenter Study of Hydroxyurea in Sickle Cell Anemia trial, no association between HU use, whether it be the female or male patient, and teratogenic effects or neonatal abnormalities was observed.

Although studies have shown HU treatment to have effects on sperm parameters, some parameters can be reversed. In one study, a 35-year-old male with sickle cell anemia had azoospermia but returned to normal spermatogenesis within 6 months after ceasing HU treatment. His wife conceived within 4 months after his stopping the treatment, giving birth to a healthy baby. Recovery of spermatogenesis was also seen in a patient being treated with HU for polycythemia rubra vera. After >10 years of treatment, he was azoospermic. Upon cessation of treatment, sperm count had returned to normal within 3 months.

Genotoxicity

In patients receiving HU for sickle cell disease, a significant increase in the number of micronuclei was observed and correlated with treatment length and the final dose. Additionally, a positive association was found between DNA damage and the mean dose and a negative association between DNA damage and treatment length. Patients with polycythemia vera developing into terminal acute myeloid leukemia or myelodysplastic syndrome had clonal chromosome abnormalities. Epigenetic changes and alterations in miRNA expression in response to HU treatment have been recently described. Compared to these studies, a recent evaluation showed that HU treatment did not induce chromosomal damage formation in children treated for up to 12 years. In another study, no significant genotoxic effects (e.g., chromosome breaks, chromatid breaks, and illegitimate VDJ recombination event) were noted in infants treated with 20 mg/kg/day HU for two years when compared to control patients.

Other Data

Mutational status has been shown to be associated with the effects produced by HU. The relationship between the presence of the *JAK2V617F* mutation and HU effect on platelet and immature platelet count, percentage of immature platelets, and percentage of highly fluorescent immature platelet fraction was evaluated. A total of 46 essential thrombocythemia and 38 polycythemia vera patients were evaluated; 23 essential thrombocythemia and 18 polycythemia vera patients taking HU. In patients negative for the mutation, there was no difference between HU-treated and non-treated groups. Comparatively, in patients positive for the mutation, HU significantly decreased platelet and immature platelet count.

Clinical Trials

A total of 96 clinical trials were identified (as of April 12, 2010). Of these, 76 were closed (i.e., no longer seeking new volunteers): 25 were active and not recruiting, 43 were completed, 7 were withdrawn/terminated, and 1 was recruiting by invitation only. Several studies were focused on children with sickle cell anemia. These studies are or were evaluating the long-term effects of HU, the efficacy of HU in treating sickle cell anemia in this population, and its ability to prevent or reverse chronic organ damage, the efficacy of HU in reducing strokes, and its efficacy in treating secondary pulmonary hypertension.

A study in 27 patients with types II and III spinal muscle atrophy was recently completed. Patients treated with HU initially consumed 7.5 mg HU/kg/day; monthly increases of 2.5 mg/kg/day were performed up to a maximum concentration of 15 mg/kg/day. Low absolute neutrophil count was 4% in the HU group compared to 2% in the placebo group. No significant hepatic or renal toxicity was noted. Motor unit number estimation was significantly higher in patients treated with HU. A trend to increased scores in Gross Motor Function Measurement subscales was also noted in the HU group.

Chemical Disposition, Metabolism, and Toxicokinetics

HU clearance is primarily via the kidneys. In sickle cell anemic adults with normal renal function, intake of a commercially available HU capsule (15 mg/kg) had a maximal concentration (C_{max}) of 28.32±11.0 µg/mL, area under the curve (AUC) of 81.66±15.5 µg·hr/mL, and half-life ($T_{1/2}$) of 3.14±0.9 hours. According to a phase 1 pharmacokinetics study, HU tablets (1000 mg) and capsules (500 mg) caused no significant differences in several parameters when measured in children and adults with sickle cell disease. Children were given HU tablets (mean dose: 21.4 mg/kg/day) for 14 days. Adults were in a randomized crossover study comparing tablets to capsules (mean dose: 20.8 mg/kg/day [capsules]) for 8 days each with a 2- to 4-week break. The difference in the renal excretion between children and adults was suggested to be due to the increase of glomerular filtration rate in adults.

In a study in which younger children with sickle cell anemia were orally administered HU (20 mg/kg), first-dose pharmacokinetic parameters were 19.81±5.8 µg/mL for C_{max}, 68.82±11.5 µg hr/mL for AUC, and 2.36±0.99 hours for $T_{1/2}$, which were lower than those of sickle cell anemic adults. In addition, infants ≤15 months were observed to have a shorter $T_{1/2}$ (2.1 versus 2.8 hours) and lower 8-hour predicted measureable HU concentration (1.2 versus 2.1 µg/mL) than children aged 16-18 months. In another study, pediatric patients were administered HU (20 mg/kg). The apparent oral clearance of HU was 0.252±0.080 L/hr/kg, slightly higher than that published for adults, and the median peak time was 0.55 hours (0.467-2.2 hours). Significant pharmacokinetic interindividual variability was seen (30.3% for C_{max}, 26.0% for $T_{1/2}$, 46.7% for apparent oral clearance, and 61.6% for apparent volume of distribution). In addition, when the patients were grouped on the basis of time to peak concentration, rapid and slow absorption was observed. In children with rapid absorption, the predicted median time to peak concentration, based on a linear one-compartment model, was 18 minutes. They also had higher median C_{max} (74% more) and median AUC (33% more) than those with slower absorption.

In a nine-year-old female administered HU (500 mg), a maximal plasma concentration of HU was achieved approximately one hour after administration (30-35 mg/L). Plasma concentration decreased in a time-dependent manner until the end of the study.

Population pharmacokinetic and pharmacodynamic models were developed based on data from clinical studies using adults that were receiving HU to assess the exposure-efficacy relationship and their variability. The models were also used to assess two different dosing regimens. The developed models were two-compartment models with first-order absorption and elimination. Based on the model, the pharmacokinetics was estimated to be linear. Additionally, it was proposed that the variability in response to HU was associated, in part, to the pharmacokinetics and pharmacodynamics of the chemical. Studies suggested that the steady-state value of the mean corpuscular volume at 3 months was not predictive of the fetal hemoglobin percentage value at 26 months, further suggesting that the fetal hemoglobin value was a more appropriate biomarker for monintoring HU treatment. Simulations using the model showed that continuous HU dosing led to a stronger response than intermittent HU dosing.

Toxicological Data

Chronic exposure, initiation/promotion, and cogenotoxicity studies were not located.

Chemical Disposition, Metabolism, and Toxicokinetics

Studies demonstrated that HU is distributed throughout the body. In mice, urea was identified as the main metabolite in urine. The elimination half-life was <0.5 hour in rodents. In a recent study, male Sprague-Dawley rats were injected with 0.5 mL radiolabeled HU as a bolus dose or 0.5 radiolabeled HU followed by short infusions (total volume = 1.9 mL) through a catheter. Results showed that overall radiolabel levels were lower in the brain compared to plasma levels. Calculated distribution and elimination half-

lives were 1.7 and 171 minutes, respectively. The brain-to-plasma ratio at 60 minutes post-administration ranged from 0.13 to 0.18.

HU was not metabolized by human liver microsomes, in the presence or absence of nicotinamide adenine dinucleotide phosphate (NADPH). Additionally, HU was not a substrate for P-glycoprotein *in vitro*. Comparatively, *in vitro* incubation of HU with rat liver homogenates increased nitrate and nitrite formation, which decreased with the addition of NADPH. Similar to *in vitro* human studies, HU was not metabolized by rat liver microsomes in the presence or absence of NADPH.

Acute Exposures

Oral LD_{50} values in mice and rats were 7330 and 5760 mg/kg, respectively. Intraperitoneal and intravenous LD_{50} values in mice and rats were >2300 mg/kg.

Short-Term and Subchronic Exposures

Overall, doses that exceeded clinical levels produced cardiovascular and hematological adverse effects in some laboratory animals [species not provided]. Bone marrow hypoplasia, pulmonary congestion, and mottling of lungs were noted in rats. Hepatic cell damage with fatty metamorphosis was noted in rats treated with doses >1200 mg/kg/day for ≥37 days.

Synergistic/Antagonistic Effects

Administration of the cyanobacterium *Spirulina maxima* (SP) antagonized HU-induced teratogenesis in CD-1 mice. In dams treated with HU alone, there were 78 viable embryos from a total of 152. Of these, 53 were identified as having developmental abnormalities (e.g., open neural tube and retarded hind and forelimb buds growth). Administration of SP or SPE significantly increased the number of viable embryos at all doses tested and decreased the number of embryos with developmental abnormalities at ≥500 mg/kg and ≥100 mg/kg, respectively. Dimethyl sulfoxide was also shown to protect embryos from the teratogenic effects of HU.

Calcium channel blockers diltiazem or verapamil enhanced inhibition of cell growth induced by HU in a human intraosseous malignant meningioma cell line. At higher HU concentrations, the calcium channel blockers did not enhance cell growth inhibition when compared to HU treatment alone. A similar effect was noted in a primary cell culture prepared from a human sphenoid wing benign meningioma. [Note: Compared to studies in the cell line, the effect of increasing concentrations of HU alone on cell growth was not graphically provided.] *In vivo* studies, using the mouse flank xenograft model, yielded similar results; addition of the calcium channel blockers enhanced decreased tumor volume. [Note: Animals implanted with malignant meningioma and treated with HU only were terminated on day 36 while other animals were terminated on day 56. Therefore, an exact comparison of the additive or synergistic effect of calcium channel blockers on HU effects is incomplete.]

Co-exposure of K562 cells, a human chronic myeloid leukemia cell line, to celecoxib and HU downregulated cyclooxygenase-2 mRNA levels, produced significant growth inhibition and increased apoptosis. The observed effects for co-incubation were greater than the effects observed when either chemical was administered separately. In HL-60 and T24 cells, HU blocked the azacitidine and decitabine-mediated inhibition of DNA methylation in a dose-dependent manner. This antagonistic effect only was observed when cells were exposed to the chemicals concomitantly. HU also antagonized estradiol induced antagonism of Fas ligand (FasL) induced apoptosis in cultured bovine granulose cells. In the absence of other chemicals, HU had no effect on FasL-induced apoptosis while estradiol antagonized the observed effect. When the two agents were combined, the level of apoptosis was similar to that observed in control and HU-treated cells.

Cytotoxicity

HU inhibits ribonucelotide reductase, which results in S-phase cytotoxicity. *In vivo* studies have also demonstrated that HU is cytotoxic after intraperitoneal (i.p.) injection. In ReNcell CX cells, HU decreased BrdU incorporation and cellular viability. HU (1 and 2 mM) significantly increased the apoptotic index in HUVEC cells. Comparatively, addition of laminar shear stress inhibited HU-induced apoptosis. Proliferative activity was not affected by addition of 1 mM HU to cells.

Reproductive and Teratological Effects

HU was a developmental toxicant in rats. Limited reproductive studies suggested that HU impairs spermatogenesis in rats and mice. Four recent studies, three in Chinese, support results from previous studies of HU effects on male reproduction. Intraperitoneal injection of 400 mg HU/kg to male Wistar rats significantly decreased Bcl-2 expression in testes after treatment and increased expression of Fas and Fast. In a separate study, HU administration by i.p. injection for 5 days increased the formation of DNA lesions in testicular cells. Male Wistar rats injected with HU (100-400 mg/kg) showed evidence of DNA damage in a dose-dependent manner.

Transgenic sickle cell mice were treated with 25 mg HU for 28 or 56 days via oral gavage. Testis weight was decreased 40% and 64% after 28 and 56 days of treatment, respectively; body weight was not affected. After 56 days of treatment, testis dimensions were decreased 52% and seminiferous tubule atrophy and Leydig cell prominence were noted. HU treatment also significantly decreased epididymal weight, sperm motility, and sperm density after 56 days of treatment. Significant decreases in plasma testosterone levels were noted after 28 and 56 days of treatment.

Recent studies also supported previous conclusions that HU effects embryonic development. One study reported that HU significantly inhibited proliferation and differentiation of midbrain cells harvested from 13-day-old rat embryos. In another study, fetuses exposed to HU *in utero* showed numerous skeletal variations (structural changes that occurred within the normal population) and malformations (changes that are likely to affect survival or development). The no observed effect level (NOEL) for observed variations was <250 mg/kg while the NOEL for observed malformations was 250 mg/kg.

Oral administration of HU for 28 days to female C57BL/6J significantly decreased ovarian weight, serum estradiol concentrations, and ovulation rate. *In vitro* studies showed that continuous treatment of 2-cell embryos with HU decreased development to blastocyst stage. Intermittent HU treatment also decreased development to the blastocyst stage, but to a lesser extent than observed with continuous treatment.

Carcinogenicity

No new carcinogenicity studies were located. One study showed that HU increased the incidence of mammary tumors in rats after i.p. injection with 125–250 mg/kg three times per week for 6 months. A separate study showed that pulmonary tumor formation was not affected by i.p. administration of increasing doses of HU for up to 1 year. In 2000, the International Agency on Research for Cancer stated that "Hydroxyurea is not classifiable as to its carcinogenicity to humans (Group 3)."

Genotoxicity

No new genotoxicity studies were located. HU was shown to be mutagenic in bacteria, fungi, protozoa, and mammalian cells [strain and/or species not provided]. Clastogenic effects have been noted *in vitro* in hamster cells and human lymphoblasts and *in vivo* in rodents. Recent studies of DNA damage in V79 Chinese hamster cells using the Comet assay showed that HU inconsistently increased tail moment at doses ranging from 50 to 500 μM after 18 hours. At the same concentrations and incubation time, HU increased chromosomal aberration formation; significant increases were noted at concentrations ≥100 μM. Chromatid breaks and exchanges were observed.

HU also produces epigenetic effects. HTB-54 lung human epidermoid carcinoma cells were incubated with HU for 24 or 48 hours. During the 24-hour incubation period, increased DNA hypermethylation was observed (>150% of control values at the highest concentration). Increased DNA hypermethylation was also observed at lower concentrations when the incubation period was increased.

HU induced micronuclei in Chinese hamster lung fibroblasts at doses 3.1-25 μg/mL and in thymidine kinase$^{+/-}$ human lymphoblastoid TK6 cells at 10 and 2500 μg/mL. HU treatment significantly increased copy number variants (CNV) in culture normal human fibroblasts; deletions and duplications were both observed. Analysis indicated that the increased CNV occur throughout the genome.

Fibroblasts and lymphoblasts obtained from patients with spinal muscular atrophy were treated with HU for 24 and 48 hours. Treatment with HU caused the greatest increase on FL-*SMN* transcripts at 48 hours. According to FL-*SMN* mRNA and SMN protein in fibroblasts, there were two responders for HU. Lymphoblasts treated with HU showed an increase in FL/Δ7 ratios. After 48 hours, viability of spinal muscular atrophy cells was decreased by 15-20% in fibroblasts and by 20-30% in lymphoblasts. A one order of magnitude dose increment caused cellular viability decreases of 20% and 40%, respectively.

Immunotoxicity

No immunotoxicity data were located. However, a recent microarray study showed that HU treatment of a human vascular endothelial cell line upregulated pro-inflammatory cytokines (e.g., RANTES, monocyte chemotactic proteins, macrophage inflammatory protein-3a, interleukin (IL)-1a, IL-1b, IL-6, and IL-8). Genes were upregulated 2- to 30-fold. A similar upregulation of gene expression was also noted in primary cultures of human umbilical vein endothelial cells (macrocirculation) and human pulmonary microcirculation endothelial cells.

Other Data

Vascular Effects

HU has been associated with acral erythema and leukocytoclastic vasculitis. In human endothelial cell lines TrHBMEC and EA-hy 926, HU significantly decreased expression of vasoconstrictor peptide endothelin-1 and vascular cell adhesion molecule. Comparatively, it upregulated expression of membrane-bound intercellular cell adhesion molecule 1, as well as the soluble form.

Mechanisms of Action

Proposed mechanisms of action associated with the therapeutic effects associated with sickle cell anemia treatment include upregulation of fetal hemoglobin synthesis; reduction in neutrophils, monocytes, and reticulocytes; effects on the sickle cell membrane, adherence molecule expression, or vascular reactivity; erythrocyte cation transport; nitric oxide formation; erythropoietin production; and/or red blood cell deformability. HU treatment of a patient with sickle cell anemia caused upregulation of transcriptional and translational regulatory genes (e.g., EGR-1). The authors proposed that these genes played a role in the therapeutic effects on sickle cell anemia.

Structure-Activity Relationships

For each GeneGo quantitative-structure activity relationship (QSAR) model, a QSAR value was calculated. For non-binary models, the calculated values ranged between two threshold values to be classified as active in the model. These threshold values corresponded to the negative logarithm of the activity for the most active compound in the training set and the negative logarithm of 50 μM (-1.7). For binary models (e.g., AMES mutagenicity binary model), the definition of an active chemical is model dependent. In addition to the QSAR value a Tanimoto similarity percentages (TP) was calculated which indicates the similarity percentage of HU to the most-similar compound in the training set.

Overall, the evaluated QSAR models predicted carcinogenic and hepatotoxic effects. Based on the predicted targets, HU is proposed to effect one-carbon metabolic process and deoxyribonucleotide biosynthetic and metabolic processes.

Absorption, Distribution, Metabolism, and Excretion QSARs

Three metabolites, identified as first-pass conjugated metabolites, were predicted. The metabolites were acylated, methylated, or glucuronidated conjugates of HU. The CYP450 models, overall, predicted that HU would have some affinity for many of the evaluated isozymes. However, the TP values were <20% for all the models. Models that evaluated the inhibitory activity of HU and its metabolites yielded similar results; compounds were identified as potential inhibitors CYP2D6 and CYP3A4 however, TP values were <20%. Predicted inhibition of human soluble epoxide hydrolase was the one exception, low inhibition activity by HU was predicted. Models of the Phase 2 metabolism enzymes predicted that HU would have affinity for the human sulfotransferase 1A1. However, as mentioned for the CYP models, the TP value was low. Models predicted that HU could enter the brain and has low protein binding potential to human serum albumin; the TP values for all predicted effects were <25%. The acetylated and methylated HU metabolites were also predicted to have some brain-penetrating ability.

Therapeutic Activity QSARs for HU

Of the 25 models evaluated, one predicted that HUwould be active (calculated value >0.5) and the TP value was >50%; anticancer activity was predicted for HU.

Toxic Effects QSARs for HU

Numerous toxic effects (calculated value >0.5 and TP value >50%) were predicted for HU. HU was predicted to be carcinogenic in mice and rats and hepatotoxic. HU was predicted to have mutagenic potential in the AMES mutagenicity binary model (0.6 [1 defined as mutagenic], TP = 100). In the general cytotoxicity model for log growth inhibition in MCF7 model, where log GI50 from 6 to8 is defined as toxic and values less than 3 are less toxic, a predicted value of 4.65 was determined (TP = 100). The model for general toxicity, based on log Maximum Recommended Therapeutic dose (mg/kg body weight/day), yielded a value of 0.82 (TP = 52.94); chemicals >0.5 cutoff value are classified as less toxic.

Possible Targets for HU and Metabolites

Most of the identified targets were based on literature information regarding the activity of HU itself. Several members of the carbonic anhydrase family were shown to be HU targets, including carbonic anhydrase II, carbonic anhydrase IV, carbonic anhydrase I, carbonic anhydrase, and carbonic hydrase VA. These enzymes are involved in the reversible hydration of carbon dioxide. As is known from previous studies, HU also inhibits the ribonucleotide reductase enzyme. No targets for the predicted metabolites were provided.

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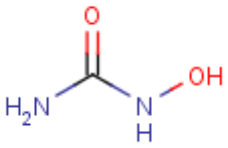
Tables:

1.0 Basis for Nomination

Hydroxyurea (HU) was nominated by the National Institute of Environmental Health Sciences for toxicological testing based on long-term safety concern when used as therapy for sickle cell anemia. HU is approved for treatment of sickle cell disease and certain cancers in adults. However, it is being used increasingly in young children and infants for treatment of sickle cell disease. There is limited information on the potential long-term consequences of HU use in infants, children, and adults. The need for multi-generation experimental animal studies were identified as a critical data need by the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR; renamed the Office of Health Assessment and Translation) Expert Panel. These studies are proposed to assess the long-term effects of prenatal and postnatal exposures on postnatal development.

2.0 Introduction

Hydroxyurea
[127-07-1]



HU was initially used 25 years ago in a clinical study of adults with sickle cell disease (Ware and Aygun, 2009). Since then, it has become the only approved disease-modifying therapy for sickle cell disease (Lanzkron et al., 2008). It has also been approved for melanoma, resistant chronic myelocytic leukemia, and recurrent, metastatic, or inoperable ovarian carcinoma, as well as for concomitant use with irradiation therapy for the treatment of primary squamous cell carcinomas of the head and neck, excluding the lip (Bristol Myers Squibb Co., 2006).

This summary and studies discussed within serve as a supplement to the NTP-CERHR Monograph on the effects of HU on human reproduction and development published in 2008 (CERHR, 2008).

2.1 Chemical Identification and Analysis

Hydroxyurea ($CH_4N_2O_2$; mol. wt. = 76.05) is also called:

Hydroxycarbamide
Biosupressin
Carbamohydroxamic acid
Carbamohydroximic acid
Carbamoyl oxime
Carbamyl hydroxamate
Droxia
HU
Hydrea
Hydroxylamine, N-(aminocarbonyl)-
Hydroxylamine, N-carbamoyl-
Hydura
Hydurea
Idrossicarbamide
Idrossicarbamide
Litalir
N-Carbamoylhydroxylamine
N-Hydroxyurea
Onco-Carbide
Oxyurea
Urea, hydroxy-

PubChem CID: 3657
InChI: 1S/CH4N2O2/c2-1(4)3-5/h5H,(H3,2,3,4)
SMILES: C(=O)(N)NO

Sources: ChemIDplus (undated); PubChem (undated)

High performance liquid chromatography (HPLC) has been widely used to determine the presence of HU. Electrochemical or ultraviolet (UV) detection has been used by numerous groups to identify underivatized HU. Detection of a derivatized form of HU has also been described (Kettani et al., 2009 [PMID:19144580]). A recent method described the spectrophotometric measurement of a sample with HPLC. Biological samples were centrifuged to remove proteins. The supernatant was then spiked with an internal standard and then combined with an acid and color reagent. After incubation, the colored reactant was then injected on the HPLC coupled to a UV detector (449 nm). The method was linear up to 1000 µM concentration and the limit of quantification was 7 µM (Sassi et al., 2010 [PMID:19817872]).

Gas chromatography/mass spectrometry (GC/MS) methods have also been described for the identification of HU. It was used for the evaluation of HU in human plasma using a new sample preparation technique. Proteins were removed from plasma samples containing an internal standard using a hexane/ethanol mixture. The lower organic/aqueous phase was extracted and evaporated to dryness. *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane (100:1, v/v) was added to silanize HU. Samples were then analyzed by GC/MS after electron-impact in the selected ion mode; monitored ions were *m/z* 277, 292, 234, and 219. The intra- and inter-day coefficients of variation were 5.3% and 7.7%, respectively. Linearity was noted to occur up to at least 100 mg/L (Kettani et al., 2009 [PMID:19144580]).

Eight pharmaceutical preparations of HU from seven manufacturers were evaluated for concentration and activity. They were manufactured in the United States, Italy, India, Germany, and Korea. Spectrophotometric chemical analysis indicated minimal variability in the prepared solutions. The average concentration of a theoretical 100 µM HU solution was 99 ± 6 µM. Functional analysis, *in vitro* inhibition of T-cell proliferation, was also similar among all test samples (Harrod et al., 2008 [PMID:18569844]).

2.2 Physical-Chemical Properties

Property	Information	Reference(s)
Physical State	White, crystalline powder	HSDB (2004)
Odor		
Boiling Point (°C)	Decomposes	HSDB (2004)
Melting Point (°C)	133-142	Registry (2010)
Flash Point (°C)		
Vapor Pressure (mm Hg)	2.43×10^{-3} @ 25°C (estimated)	HSDB (2004)
Density (g/cm^3)	1.457 ± 0.06 @ 20°C/760 Torr*	Registry (2010)
Solubility	In water and hot alcohol; insoluble in ethanol and benzene	HSDB (2004)
Octanol-water partition coefficient (log K_{OW})	-1.80	HSDB (2004)
Log P	-1.800 ± 0.187*	Registry (2010)
Bioconcentration Factor	1.0 @ 25 °C (pH 1-10)*	Registry (2010)

*calculated properties using Advanced Chemistry Development (ACD/Labs) Software V8.14 (©1994-2010 ACD/Labs).

2.3 Commercial Availability

Bristol Myers Squibb Company produces two brand name drugs whose active ingredient is HU, HYDREA® and DROXIA®. Both drugs are available by prescription and are for oral use. Dosages range from 200 to 500 mg. Two companies currently produce unbranded (generic) equivalents of the Hydrea 500 mg capsules, Barr Laboratories Inc. and Par Pharmaceutical Inc. (U.S. FDA, 2010).

Chattem Chemicals, Inc (Chattanooga, TN) and DSM Catalytica Pharmaceuticals, Inc. (Mountain View, CA) were listed as manufacturers of HU in the Hazardous Substances Data Bank file for HU (HSDB, 2004). Additional U.S. producers of HU include Santa Cruz Biotechnology, Inc. and Hongda Group Limited. Simagchem Corporation; Jinan Wedo Industrial Co., Ltd.; NSTU Chemicals Hangzhou Co.; Ningbo Pangs Chem International Co., Ltd.; Hangzhou Meite Chemical Co., Ltd.; and Hallochem Pharma Co., Ltd., all of China, and Jai Radhe Sales of India are listed as global producers (BuyersGuideChem, 2010).

U.S. suppliers of HU include Spectrum Chemical Manufacturing Corporation, AK Scientific, Inc., Interchem Corporation, and Research Organics Inc. Global suppliers include Chemos GmbH of Germany and Jinan Haohua Industry Co., Ltd.; Shanghai AOKChem Group, Ltd.; Suzhou Rovathin Foreign Trade Co., Ltd.; and Leap Labchem Co., Ltd., all of China (BuyersGuideChem, 2010).

3.0 Production Processes

HU can be synthesized by reacting hydroxylamine hydrochloride with potassium cyanate in aqueous solution or calcium cyanate with hydroxylamine nitrate in absolute ethanol (Budavari, 1996; Graham, 1955 pat.). An alternate synthetic pathway was described in a U.S. patent issued in 1955. In this method, a quaternary ammonium ion exchange resin is reacted with a sodium cyanate to convert the resin to the cyanate form. The resin is then reacted with hydroxylamine hydrochloride to produce HU (Graham, 1955 pat.).

4.0 Production and Import Volumes

No data were located.

5.0 Uses

HU is the only approved disease-modifying therapy for sickle cell disease (Lanzkron et al., 2008). DROXIA® has been approved for use in reducing the frequency of painful crises and the need for blood transfusions in adults with sickle cell anemia with recurrent moderate to severe painful crises (Bristol Myers Squibb Co., 2007). HYDREA® has been indicated for the use of treating specified cancers; melanoma, resistant chronic myelocytic leukemia, and recurrent, metastatic, or inoperable ovarian carcinoma. It was also approved for concomitant use with irradiation therapy for treatment of primary squamous cell carcinomas of the head and neck, excluding the lip (Bristol Myers Squibb Co., 2006).

HU has also been used for the treatment of essential thrombocythaemia, spinal muscular atrophy, intracranial meningiomas, and recalcitrant psoriasis (Birgegard, 2009; Darras and Kang, 2007 [PMID:18025935]; Dashti et al., 2009 [PMID:19482663]; Dourmishev and Dourmishev, 2008 [PMID:18613806]). Several clinical trials have also evaluated or are evaluating the efficacy of

HU, alone or in combination with other drugs, for the treatment of a variety of medical conditions. These conditions include malignant glioma, spinal muscle atrophy, human immunodeficiency virus, chronic myelogenous leukemia, locally advanced nasopharyngeal carcinoma, advanced mouth cancer, and advanced stomach cancer (ClinicalTrials.gov, 2010; Wong et al., 2006).

Several studies of the effects of HU in children with sickle cell disease are provided in **Section 9.1.1**.

6.0 Environmental Occurrence and Persistence

Data were not evaluated for this report.

7.0 Human Exposure

Data were not evaluated for this report.

8.0 Regulatory Status

DROXIA® was originally approved for use in the treatment of sickle cell disease in 1998 (Lanzkron et al., 2008).

The Best Pharmaceuticals for Children Act requires identification and prioritization of approved drugs for which studies in children are needed. Under this act, HU was initially listed in 2006 (NIH, 2006). In 2004, the U.S. Food and Drug Administration (FDA) requested that that Bristol Myers Squibb Company conduct studies regarding treatment of sickle cell disease in children; the request was declined. Accordingly, the FDA referred the request to conduct these studies to Foundation for the National Institutes of Health (U.S. FDA, 2005).

9.0 Toxicological Data

9.1 General Toxicology

9.1.1 Human Data

In general, this section provides data from studies published between January 2007 and the present. The studies serve as supplementation to the NTP-CERHR Monograph (CERHR, 2008). Information in the accompanying table (**Table 1**) is presented in order of disease state, with data for children followed by that for adults, where applicable.

Efficacy Studies

Although HU is the only approved disease-modifying therapy for sickle cell disease in adults, it is being used for the treatment of sickle cell disease also in children (e.g., see Strouse et al. [2008] for a review of its efficacy). Additionally, HU is used for the treatment of other disorders. For example, it is used for the treatment of the rare hematologic disease polycythemia vera. In 1638 patients (European Collaboration on Low-Dose Aspirin in Polycythemia prospective study), HU did not enhance the risk of leukemia when compared to patients treated with just phlebotomy (hazard ratio = 0.86, 95% confidence interval 0.26-2.88, $p = 0.8$). However, risk was significantly increased with the combination of HU with another chemical such as bisulphan or an alkylating agent (Finazzi et al., 2000, 2005; both cited by Finazzi and Barbui, 2007).

Examples of current studies reporting HU treatment are presented in **Table 1**.

Table 1. Selected Studies in Humans Using Hydroxyurea for Treatment

Study Population	Disease	Treatment	Effects	Reference
Efficacy and Safety Studies				
193 patients (96 received HU; 97 received placebo); mean age = 13.6 and 13.5 months, respectively; 170 completed study to at least 18 monthe; 167 fully completed study 91 HU treated and 88 placebo analyzed [BABY HUG Clinical Trial]	sickle cell anemia 93-94% with HbSS	20 mg/kg/day for 2 years	Splenic function at end of the study was not signficiantly different between the two groups. Similarly, DTPA glomerular filtration rate was not different between the two groups at the end of the study. Seconday measures of splenic function (e.g., liver count ratios and pit counts) and renal function (e.g., urine osmolality) were improved by HU-treatment. HU treatment was also associated with a reduced number of vaso-occlusive events and improved hematologic counts (e.g., hemoglobin and white blood cell count). Transient neutropenia was noted.	Wang and Thompson (2010 abstr.); Wang et al. (2011)
14 children; 11M, 3F; average age = 35 months (21-53)	sickle cell anemia HbSS (one also had 2-gene deletion α-thalassemia trait)	initial dose = 20 mg/kg/day; dose increased by 5 mg/kg/day every 8 weeks to a maximum tolerated dose or 30 mg/kg/day; follow-up of 2 years	Significant increases in Hb concentration, mean corpuscular volume, and % fetal Hb were observed. Significant decreases in reticulocyte count, absolute neutrophil count, and serum bilirubin were also noted. Glomerular filtration rate was not increased. Transcranial Doppler velocities were decreased; new central nervous system changes were not noted. Hematological toxicity occurred 23 times in 9 patients: 11 neutropenia, 6 anemia, 4 thrombocytopenia (in one child), and 2 combined cytopenias. During evaluation period, 3 patients each required a single transfusion; two episodes of ACS occurred; and there were two hospitalizations for painful vaso-occlusive events.	Thornburg et al. (2009) [PMID:19061213] [See also Zimmerman et al. (2007) for similar results on transcranial Doppler velocity.]

Study Population	Disease	Treatment	Effects	Reference
21 children: 9M, 12F; median age = 3.4 years (2.6-4.4); all were African-American. All patients (6 months-24 months of age) had previously completed a 2 year study of HU treatment.	sickle cell anemia 20 had homozygous sickle cell anemia and 1 had Hb $S\beta^0$-thalassemia	initial dose = 20 mg/kg/day; dose increased by 5 mg/kg/day every 6 months until maximum dose of 30 mg/kg/day. One patient required dose reduction to 27.5 because of neutropenia. Patients were followed for up to 4 years.	Mean Hb concentration, MCV, Hb F, and F-cell values were significantly higher and mean platelet count, reticulocyte counts, and white blood cell count were significantly lower during the treatment year 3 and 4, when compared to untreated children. Mild to moderate neutropenia, thrombocytopenia, severe anemia, and acute splenic sequestration were observed during the course of study; one death (from sepsis) was noted. Eight acute chest syndrome and 36 pain events were recorded. In 14 patients, spleen activity was assessed at baseline, year 2 and year 4: 3 (21.5%) had normal splenic uptake, 3 (21.5%) had decreased uptake, 2 (14%) had markedly decreased uptake, 6 (43%) were functionally asplenic; percentage of patients functionally asplenic was significantly lower than the expected incidence (94%) in untreated patients. No brain atrophy was noted. There was improved growth rate compared to untreated patients.	Hankins et al. (2005) [PMID:16172253]
9 patients: 4M, 5F; median age = 14 years	sickle cell anemia HbSS (8 patients) and HbSothalassemia (1 patient)	Patients initially received liquid HU at 20 mg/kg/day for 2 years, then underwent dose escalation to 30 mg/kg/day. Median dose of HU 10 years after dose escalation was 26 mg/kg/day. Patients had almost 13 years of continuous HU therapy.	HU treatment produced long-term increases in hemoglobin and MCV. [ILS Note: Internet search indicates that MCV most likely refers to mean corpuscular volume.] Mean percentage of fetal hemoglobin was >20%. Height and weight growth rates were ~50%. No neurological impairment (based on the fact that there was no history of repeated grades) was observed. Menarch occurred at a median age of 12. [ILS Note: It was stated earler in the abstract that five females were evaluated, but the results states that five of six girls reached menarche.] Two patients developed transient neutropenia. Limited adverse events were noted.	Hankins et al. (2010 abstr.)

Study Population	Disease	Treatment	Effects	Reference
47 Tunisian children (sex n.p.); median age = 12.5 years	sickle cell disease (including 27 homozygous and 20 double heterozygote sickle cell disease-S/β-thalessemia	average of 20 mg/kg/day (14-30) for an average of 52 months (18-81)	Treatment was well tolerated by the patients. There were significant decreases in the number of days of hospitalization per patient and per year (29.3 to 3.2), white blood cell rates (14,914 to 8464/mm^3), polynuclear neutrophils (6799 to 3486 /mm^3), and platelets (508,666 to 293,500/mm^3) and significant increase in fetal Hb rates (3 to 30%), Hb (7.8 to 9.6 g/dL), and average blood cell volume (79.1 to 100.3 fl).	Mellouli and Bejaoui (2008) [PMID:18164913]
123 Algerian patients (sex n.p.); medium age = 15 years (3-25)	46 with sickle cell disease	19.5 mg/kg/day (14-24); medium follow-up of 82 months	There was a significant decrease in the annual rate of vaso-occlusive crisis, acute chest syndrome, blood transfusion, and day hospitalizations. In 33 patients, stopped blood transfusions had a mean hemoblogin of 1.4 g/dL (0.8-3.3). In 7 patients, ferritin levels decreased from 3500 to 870 ng/mL with deferoxamine and phlebotomy. One person had a stroke, 1 recurrent priapism, 4 hip osteonecrosis, and 2 died. Five patients exhibited growth retardation. HU treatment reduced annual costs by ~85%.	Bradai et al. (2009 abstr.)
91M (41 with sickle cell, 50 control); average sickle cell age = 12.81 years	sickle cell disease	starting at 15 mg/kg/day; treatment was at least for 2 years	Compared to control population, weight, height, % body fat, body fat weight, and lean body weight were significantly different in children with sicke cell disease that were being treated with HU. Right hand and leg strength, and flexibility were significantly decreased compared to controls. Heart rate changes during exercise were not significantly different between the two groups. Blood analyses showed that hemoglobin concentration was significantly lower in sickle cell disease children compared to controls. However, these levels were also significantly greater than compared to prior initiation of HU.	Wali and Moheeb (2011) [PMID:21083357]

Study Population	Disease	Treatment	Effects	Reference
152 patients, HU treatment: 75M, 77F; mean age = 30.6 (18-59) 147 patients, placebo: 71M, 76F; mean age = 29.8 (18-54) 135 of 299 patients completed the full 2-year follow-up (mean = 21 months)	sickle cell anemia	n.p. [patients enrolled from 21 sites into study almost 20 years ago]	HU reduced pain intensity (2.51 versus 2.82 placebo). This was obtained early at 6 weeks and sustained. HU also slightly reduced analgesic use (0.40 days versus 0.44 days placebo) and utilization (0.08 versus 0.11 days placebo). Patients with higher HbF treatment responses to HU had statistically significantly lower use and utilization. HbF reponse to HU was a significant indicator of pain intensity, analgeis use, and utilization.	Smith et al. (2011) [PMID:21481164]
330 patients (133 received HU; 199 treated conventionally); sex and age n.p.	sickle cell anemia 34 with HbS/HbS, 131 with HbS/β0-thalessemia, and 165 with HbS/β+-thalessemia (107 with HbS/IVSI-110 and 58 with HbS/IVSI-6)	20 mg/kg/day (15- 35 mg/kg/day); median follow-up period was 8 years for HU patients and 5 years for non-HU patients	Significant increases in total Hb and fetal HbF were observed at 6 and 12 months and at the last follow-up. There was a median fivefold increase of fetal Hb at 6 months. The frequency of severe painful crises, transfusion requirements, hospital admissions, and frequency of chest syndrome were reduced. Significant reductions in leukocyte, platelet, and reticulocyte counts and in serum bilirubin and LDH levels were also seen. Death rate was decreased in HU patients compared to non-HU patients (13/133 vs. 49/199 deaths).	Voskaridou et al. (2009 abstr.)
40 patients: 14M, 26F; mean age = 56.7 years (20-82 years) ; 15 treated with HU, 25 treated with low-dose aspirin	essential thrombocythemia	average dose: 1000 mg/day (500-2000 mg/day) ≤4 wks	No serious adverse effects were reported. HU caused a significant decrease in platelets (1015 vs. 433 $\times 10^9$/L), white blood cells (11 vs. 7 $\times 10^9$/L), and median platelet-monocyte conjugates after stimulation with adenosine diphosphate/collagen (41.67 vs. 33.68). Hb levels and red blood cell count remained stable. Note: Compared to aspirin, HU was a poor treatment for the reduction of platelet/leukocyte conjugates.	Trelinski et al. (2009) [PMID:19741509]

Study Population	Disease	Treatment	Effects	Reference
49 patients: 21M, 28F; mean age = 18.38 years (1-40), splenectomized and transfusion-dependent	major β-thalassemia	mean dose = 10 mg/kg (8-15 mg/kg) daily; dose gradually increased if no side effects observed; mean follow-up was 5 years patients treated with folate and calcium supplements 6 months prior to and during treatment	The number of transfusions decreased beginning in the first 3-4 months. The mean packed red cell transfusions one year before starting of HU was 22.75 units, which decreased to 6.02 units after treatment (P<0.01). The mean ferritin level during the first period was 2751.44 ng/mL, but it decreased to 1594.20 ng/mL after one year of HU therapy (P<0.001). The mean deferoxamine injection decreased from 84.83 to 49.46 (p<0.001). Hb levels remained steady (8.5 g/dL vs. 8.04 g/dL). HU treatment was well-tolerated. At treatment start, 8 patients experienced nausea, which ended on its own accord. HU did not cause any hematopoietic suppression, except in one patient who developed transient thrombocytopenia, which resolved after a short period of HU cessation. No malignancies, including leukemia, occurred in the follow-up.	Zamani et al. (2009)

Study Population	Disease	Treatment	Effects	Reference
152 patients, 146 evaluated at follow-up: 74M, 72F; median age = 7.27 years (1-23)	β-thalassemia major	mean dose = 16 mg/kg daily (starting dose was 10 mg/kg and increased by 1 mg/kg at 4-week intervals to maximum of 20 mg/kg or until myelotoxicity occurred); results analyzed at end of 2 years	Response to HU therapy seen at a median of 65 days (30-180) after start. After HU treatment, 60 children (41%) did not need transfusion. Mean volume of PRC transfused was 965.6 mL prior to HU. Mean height and weight increased but serum ferritin levels decreased. For children with β-thalassemia major, mean Hb was maintained at 7.6 g/dL (mean 7.5 g/dL during control period). For those with β-thalassemia intermedia, Hb was increased from 6.7 to 7.8 g/dL. Fifty-seven patients (39%) were partial responders, with >60% reduction in packed red blood cell transfusion (1246 to 474 mL, P=0.011). There was a significant increase in mean weight and height and a statistically insignificant decrease in serum ferritin levels. Twenty-nine patients (20%) were nonresponders, with 34% reduction in PRC transfusion requirement (1359 to 901 mL). There was a statistically significant increase in mean weight and height and a slight increase in serum ferritin levels. Side effects (e.g., confusion, rashes, and polyuria) were minimal. Four children developed mild myelosuppression and two developed diarrhea, which were reversed/relieved by stopping HU for one week. HU was restarted in the 6 patients and well-tolerated afterwards.	Ansari et al. (2011) [PMID:21602718]

Study Population	Disease	Treatment	Effects	Reference
123 Algerian patients (sex n.p.); medium age = 11 years (3-21)	79 with thalassemia	16.1 mg/kg/day (13-21); medium follow-up of 82 months	Severe acute toxic effects were not observed. A 50% reduction in blood transfusions was seen in 47 patients. In 36 patients, mean ferritin levels decreased from 4100 to 2100 ng/mL with deferoxamine and phlebotomy (14 patients). Four patients had portal vein thrombosis, and 3 had autoimmune anemia. Seven patients exhibited growth retardation, and 6 died (3 from cardiac deficiency [1 portal venous thrombosis, 1 sepsis, and 1 acute leukemia]). HU treatment reduced annual costs (i.e., compared to conventional treatment [e.g., blood transfusions]) by ~75%.	Bradai et al. (2009 abstr.)
84 patients; mean age = 18.19 ± 6.58 (4-37), sex n.p.	β-thalassemia intermedia	mean dose of 10 mg/kg/day for 1 year	HU treatment significantly increased hemoglobin levels. However, did not have a significant effect on pulmonary acceleration time or M-mode, Doppler and tissue Doppler images..	Amoozgar et al. (2011) [PMID:21447009]
6 children: 3M and 3F; average age = 9.8 years (8-13)	transfusion-dependent β-thalassemia major transfusions beginning at 6 months to 2 years; splenectomized at 3 years to 6 years	5 mg/kg/day (starting dose) 5 days/week, increasing at 5 mg/kg/day at intervals of 12-24 weeks until hematologic toxic effects observed; follow-up for 1, 2, and 5 years Maximal average tolerable dose was 15 mg/kg/day.	No significant adverse effects or complications were observed with HU treatment (e.g., normal total leukocyte and platelet counts). After 5 months of HU therapy, blood transfusions occurred at intervals of 4-5 weeks; after 11 months, transfusions occurred at intervals of 6-8 weeks. After 1 year, 3 patients no longer had transfusions (total Hb stabilized and increased) and 2 patients had longer transfusion periods (4-5 month intervals) with no change in Hb level. After >5 years, 3 patients still had no transfusions (Hb level steady between 100-108 mg/L). Fetal Hb levels increased but were variable in all patients. Serum ferritin levels decreased during HU treatment.	Mtvarelidze et al., (2008) [PMID:18403819]
1M; 22 years old	secondary polycythemia due to cyanotic congenital heart disease	1 g/day for >8 months	Patient had pulmonary atresia with ventricular septal defect requiring frequent phlebotomies. After 8 months of HU treatment, clinical improvement was seen. Hematocrit levels were <60% and the patient only had to endure 3 phlebotomies.	Boussaada et al. (2007) [PMID:17657936]

Study Population	Disease	Treatment	Effects	Reference
3M, 1F; 16-42 years old	secondary erythrocytosis in cyanotic congenital heart disease	10-13 mg/kg/day, increasing by 5 mg/kg/day every 8 weeks until clinical improvement [median duration of 15 months (6-24 months); median dose 15.5 mg/kg/day (7-25)	Significant reduction in erythoryctosis: Median hematocrit decreased from 71% to 62%; median red blood cell count decreased from 8.02×10^{12}/L to 5.67×10^{12}/L; increases in mean corpuscular volume and mean corpuscular Hb (medians 111 fL and 39.1 g/dL, respectively). There were slight, insignificant effects on Hb concentration, fetal Hb level, and white blood cell count. Two patients had excessive meylosuppression without symptoms or infections, with one of the two also experiencing a drop in platelet count. Lowering HU dose reversed these complications.	Reiss et al. (2007) [PMID:17506064]
1M; 12 years old	desmoid tumor	n.p.	After two excisions were performed, 18-month chemotherapy with HU was prescribed to prevent recurrence of tumor. Two years later, tumor recurrence was not seen.	Ramirez et al. (2009) [PMID:19322113]
17M, 20F; average age = 16.6 (5-41)	spinal muscle atrophy	10 mg/kg/day for initial 4 weeks, then 15 mg/kg/day 4 weeks, then 20 mg/kg/day for 64 weeks	No significant effect on motor function, strength, lung function, or serum full-length survival motor neuron mRNA in patients with type 2 and 3 spinal muscle atrophy. The only adverse effect that was significantly different between treated and control groups was neutropenia development.	Chen et al. (2010) [PMID:21172842]
1M; 15 years old	aggressive fibromatosis	20 mg/kg/day	After surgery, chemotherapy, and radiotherapy, patient was given oral HU. No side effects were noted and partial tumor response was observed within 3 months. Response persisted at an assessment 4 months later.	Meazza et al. (2010 lett.)
1F, 28 years okd	sickle cell anemia with chronic immune thrombocytopenic purpura	started on HU at 1000 mg twice daily, then lowered to 500 mg twice daily	Clinical remission of chronic immune thrombocytopenic purpura and no vaso-occlusive events for 6 months. This was then followed by an aplastic crisis. When HU dose was lowered, one admission for vaso-occlusive crisis and a remission of chronic immune thrombocytopenic purpura occurred for 7 years.	Schloemer et al. (2011 lett.)

Study Population	Disease	Treatment	Effects	Reference
Studies Reporting Adverse Effects				
158 children: 81M, 77F; average age =16.6 years The children were Black and Hispanic (Bronx, NY).	sickle cell disease with avascular necrosis of the femoral head (AVN)	n.p.	Twenty-six (16.5%; 17M and 9F) had AVN; 15 of them being treated with HU had a higher odds of having AVN compared to those not being treated with the chemical (odd ratio 3.51, 95% confidence interval 1.31, 9.38, p=0.013). This was significantly higher than the ~6% reported by the Cooperative Study of Sickle Cell Disease for comparative age groups in a prospective study.	Mahadeo et al. (2008 abstr.)
175 children: 102M, 73F; age n.p.	sickle cell disease	n.p.	Comparison of children with sickle cell disease treated and non-treated with HU (from SC Medicaid database) indicated that while treatment was not associated with development of serious adverse effects it was being administered to those already with organ-specific complications.	Tripathi et al. (2011) [PMID:20922765]
143 patients; 78 M, 65 F; median age = 21 years	thalassemia intermedia	mean dose =10.74 mg/kg/day; duration of treatment ranged from 1.5 to10 years (median duration = 5.7 years)	Adverse were noted in 30.7% treated patients and included skin hyperpigmentation, partial alopecia, maculopapular rash, headaches, dizziness, anorexia, facial erythema, tarry stool, and nausea and vomiting. None of the adverse effects resulted in discontinuation of the therapy.	Karimi et al. (2010) [PMID:20367264]
1M, 46 years old (from the Caribbean islands)	drug-induced hypersensitivity syndrome; chronic myeloid leukemia	n.p.; imatinib and HU	HU was implicated as the causal drug; onset occurred in 4 days. In addition, the patient was hypotensive and exhibited cardiac symptoms (i.e., had negative T waves on electrocardiogram and ASA hypokinesia, the left ventricular ejection fraction being 40%). He also had hepatitis and thrombocytopenia.	Ben m'rad et al. (2009) [PMID:19440116]
1 patient (sex n.p.), 62 years old	chronic myeloproliferative disease	doses n.p.; treated for 9 years	Patient developed multiple cutaneous neoplasms.	Wiechert et al. (2009) [PMID:19096810]

Study Population	Disease	Treatment	Effects	Reference
933 patients: 483M, 510F; median age = 60.5 years (20-92.7[interquartile]) 614 treated with HU: 277M, 337F; median age = 64.4 years (54.4-72.7 [interquartile])	Philadelphia chromosome-negative myeloproliferative neoplasms 476 with essential thrombocythemia, 347 with polycythemia vera, 140 with primary meylofibrosis, and 30 with unclassifiable chronic meyloproliferative disorders; for patients with HU treatment, these numbers are 309, 214, 73, and 18	first-line treatment in 523 pateints (85.2%) and as a second- or third-line treatment in 91 patients (14.8%); mean dose of 1085±390 mg	After a median treatment period of 32.1 months (10.5-74.6 [interquartile]) with a mean dose of 1085 mg: • 51 patients (8.3%) had mucocutaneous toxicity • 30 (58.5%) had painful ulcerative skin toxicity, mostly in perimalleolar area • 11 (21.6%) had oral aphthous ulcers • 10 (19.6%) had nonulcerative skin toxicity with erythema and skin infiltration After mucocutaneous toxicity, HU treatment continued at same dose in 5 patinets (9.8%), reduced in 12 (23.5%), temporarily discontinued in 7 (13.7%), and completely stopped in 27 (52.9%). After a median treatment period of 4.3 months (2.4-9.0 [interquartile]), 39 patients (76.5%) had complete resolution and 12 (23.5%) had improvement but without complete resolution.	Latagliata et al. (2011) [PMID:21692060]
1F, 68 years old	polycythemia vera	doses n.p.; treated for 8 years	Patient developed painful ulcers on lower legs, multiple hypertrophic actinic keratoses, and a squamous cell carcinoma. Ulcers disappeared within 8 weeks of discontinuation of treatment. The other symptoms were treated with cryotherapy and excision, respectively.	Hoff et al. (2009) [PMID:19756436]
386 patients: 144M, 242F; median age = 64 years (16-91) 136 treated with HU; no additional information provided	essential thrombocythemia	induction dose = 30 mg/kg/day, adjusted to maintain platelet count at $<400\times10^9$/L	Twenty-six patients experienced thrombotic events, and 6 patients experienced hemorrhagic events.	Palandri et al. (2009) [PMID:19208420]
116 patients (of 194 treated with chemotherapy); male/female ratio: 0.54; median age at diagnosis = 64.5 years	essential thrombocythemia	doses varied with patient (in 15, it ranged from 130 g for 5 months to 2574 g for 132 months); median follow-up of 104 months	After 87 months (median) from diagnosis, 13 (11.2%) patients treated only with HU developed a second malignancy (2 hematological, 11 non-hematological). In comparison, the malignancy was seen in 10 of 40 (25%) of patients treated with alkylating agents followed by HU.	Radaelli et al. (2008) [PMID:18796244]

Study Population	Disease	Treatment	Effects	Reference
Reproductive Toxicity Studies				
75M (average age = 29.8 years) and 77F (average age = 27 years); 10M and 6F having known HU usage	sickle cell anemia	n.p.; 17-year follow-up postrandomization	Outcomes of 6 pregnancies: 2 live births (1 full term and 1 premature), 3 elective abortions, and 1 spontaneous abortion. Two elective abortions and the spontaneous abortion had "probable" HU usage (i.e., known usage at conception, sometime during gestation, and at the time of delivery). For males, pregnancy outcomes of female partners: 6 live births (4 full term, 1 premature, and 1 gestational age >37 weeks but weight unknown), 2 elective abortions, and 2 spontaneous abortions. No association between HU use and teratogenic effects or neonatal abnormalities was seen. Additionally, no delays in development milestones were observed.	Ballas et al. (2009)
4M; mean age = 22.2 years (19-24); treatment initiated at age 8, 10, 11, or 16 years	sickle cell anemia	~20 mg/kg/day for years (not specified)	Two of four patients (with shorter treatment at 8 and 9 years) were oligozoospermic and had increased percentages of morphologically abnormal spermatozoa. The other two patients (treatment for 12 and 15 years) were azoospermic. All patients had increased percentages of morphologically abnormal spermatozoa.	Lukusa et al. (2009) [PMID:19437321] Additional information provided by Lukusa and Vermylen (2008).

Study Population	Disease	Treatment	Effects	Reference
4M; average age = 29.25 years (21-40) at start of HU treatment	sickle cell disease in 3 patients, polycythemia rubra vera (PRV) in 1 patient	1000, 1250, or 1400 mg/day for 8 to 126 months before semen analysis	Patient 1 (PRV, start age: 27 years): azoospermic after >10 years on HU. Three months after stopping treatment: sperm count was normal (30 million/mL) but there was a high percentage of abnormal forms (94% small or absent acrosomes) and motility was impaired (20%). Six months after resuming use of HU: patient was azoospermic again. Patient 2 (start age: 40 years): normal sperm count with reduced motility after 17 months on HU. Thirty-two months after stopping treatment: sperm count (15 million/mL), motility (21%), and morphology (94% small or absent acrosomes) were subnormal. Patient 3 (start age: 21 years): low sperm count (15 million/mL) with normal motility (58%) and morphology (50% [forms not specified]) one year after stopping 49-month HU treatment. [Results while on treatment not provided.] Patient 4 (start age: 29 years): low sperm count (4 milllion/mL) with normal motility but abnormal morphology (70-88% small or absent acrosomes) when tested at 3, 23, and 44 months during HU treatment. [Results after stopping treatment not provided.] His partner, however, became pregnant during year 3 of treatment.	Grigg (2007) [PMID:17316339]

Study Population	Disease	Treatment	Effects	Reference
44M; mean age = 25.8 years (16-48); mean age of puberty in 11 patients = 14.4 years (12-15.5)	sickle cell disease 41 homozygous SS, 1 compound heterozygote SC, and 2 compound heterozygotes for sickle β^0 thalassemia	20-30 mg/kg bw/day	In 76 samples from 34 patients ***before*** HU treatment, abnormal values (>50%) were reported in initial forward motility (83.6%) and spermatozoa morphology (64.1%). In 6 samples from 5 patients ***during*** treatment, abnormal spermatozoa concentration (100%), total sperm count (100%), initial forward motility (80%), and spermatozoa morphology (66.7%) were observed. In 26 samples from 8 patients ***after*** treatment, all previous sperm parameters were affected; in addition, vitality was affected (77% abnormal). One patient was azoospermic. Pregnancies were normal. [Authors note that semen alterations may be due to the disease and that HU treatment may worsen the changes.]	Berthaut et al. (2008)
1M, 35 years old	essential thrombocythemia	2 g/day for 3 years	Three years after beginning HU treatment and failure to conceive with wife, both testes were reported to be slightly atrophic, with bilaterally palpable vasa deferens and mild bilateral epididymitis. Semen analysis showed azoospermia. No other effects were reported (e.g., serum testosterone levels normal and no distended epididymis). Within 4 months after HU was stopped, patient's wife conceived and delivered a healthy baby; after 6 months spermatogenensis returned to normal: 0-1 sperm during treatment 61 million sperm 3 months later 200 million sperm 6 months later	Masood et al. (2007) [PMID:17333529]

Study Population	Disease	Treatment	Effects	Reference
Genotoxicity Studies				
79 children reenrolled in the Hydroxyurea Study of Long-term Effects clinical trial (HUSTLE; NCT00305175)	sickle cell anemia	maximum tolerated dose with exposure ranging from 0 to 12 years	No positive correlation between HU treatment and baseline DNA damage. Treated children had significantly lower number of chromosomal abnormalities compared to controls with no HU exposure. Similar lack of correlation was noted when specific chromosomal abnormalities (e.g., chromatid breaks) were evaluated. Length of exposure also did not induce differences in chromosomal breakage levels.	McGann et al. (2011a) [PMID:21542824]
Children enrolled in the Hydroxyurea Study of Long-term Effects clinical trial (HUSTLE; NCT00305175)	sickle cell disease	maximal tolerated dose; no additional description provided in paper	While methylation within the $^{G}\gamma$-globin promoter region was inversely correlated with fetal hemoglobin, HU treatment significantly decreased methylation at three CpG sites <5%. Comparatively, specific miRNA expression was increased after treatment.	Walker et al. (2011a) [PMID:21921042]
105 children 37 of 105 children were followed for 2 years with serial measurements	sickle cell disease	median dose = 25.0 mg/kg/day (13.9-32.4 mg/kg/day)	In a cross-sectional analysis of blood samples from 105 children (on HU for a median of 2 years), showed increased frequency of micronuclei (MN)-containing young reticulocytes and MN-containing red blood cells. Effects were seen within 6-9 months of treatment, with no further increase up 12 years of continued use. In prospective analysis of 37 children, the increase in MN-containing young reticulocytes ranged from 1.29 to 3.16-fold increases within 9 months. This observed increase was associated with increased MN-containing red blood cells and reductions in reticulocyte frequency.	Flanagan et al. (2010) [PMID:20230905]
193 infants; mean age = 13.6 months [BABY-HUG Phase III clinical trial]	Sickle cell disease	20 mg/kg/day for 2 years	Similar number of chromosome and chromatid breaks was noted at study end between HU-treated and control participants. Similarly, the number of VDJ recombination events and % of micronucleated $CD71^{+}$ retriculocytes was similar between the two groups at the end of the study. There was a significant increase in the number of chromosome breaks and % of micronucleated $CD71^{+}$ reticulocytes in treated participants between the start and end of the study.	McGann et al. (2011b) [PMID:22012708]

Study Population	Disease	Treatment	Effects	Reference
35 patients: 15M, 20F; mean age = 26.3 years (4-59) 25 of the 35 also assessed for effect of treatment length and final dose on MN frequency	sickle cell disease (hemoglobinopathy SS)	oral; 8.7-28.5 mg/kg/day (medium 26.5 mg/kg/day) for 16-161 months (median 49 months)	A significant increase in the number of micronucleated cells was observed in white blood cells (4.7 vs. 3.5 controls) in the cytokinesis-block micronucleus assay. Treatment length and the final dose affected MN frequency: as treatment length increased, MN frequency decreased, but as final doses increased, MN frequency increased. No effect on the frequency of nucleoplasmic bridges or nuclear buds was observed.	Maluf et al. (2009)
28 patients: 13M, 15F; mean age = 23.1 years (2-59)	sickle cell disease 1 with S-β-thalassemia, 2 with SC hemoglobinopathy, 25 with SS hemoglobinopathy	oral; median of 27.8 mg/kg/day for 0.8-13.4 years	A positive association between DNA damage index and mean HU dose was seen in peripheral blood leukocytes using the Comet assay. A 1.7-fold increase in DNA damage was observed at doses >27.8 mg/kg (damage index = 24 vs. 14 at lower doses). There was a significant, negative association between damage index and length of treatment (damage index = 13 for treatment ≥40 months vs. 24 at <40 months]). There was a non-significant, positive association between damage index and age (damage index = 24 at ≤20 years and 14 at ≥21 years).	Friedrisch et al. (2008) [PMID:17988936]
7 patients (sex n.p.); 42-79 years old	polycythemia vera (PV) developing into terminal acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS)	n.p.; medium time from PV diagnosis to AML/MDS relative to treatment in the polycythemic phase: 149 months (39-252)	Survival time after AML/MDS diagnosis was 1-42 months. Five patients had clonal chromosome abnormalities. Study results combined with those in the literature (n=12 patients total) show half of the patients had a -5/5q- or -7/7q- abnormality. Comparing patients treated with HU in their "multiple therapy lines" to those not using HU, no difference in abnormalities was seen.	Swolin et al. (2008) [PMID:18351338]

Study Population	Disease	Treatment	Effects	Reference
Stroke Treatment				
43 patients: 19M, 24F; mean age at first stroke = 7.0 ± 3.9 years 10 (7M, 3F) of 43 on HU therapy; assessed for efficacy in prevention of stroke recurrence	sickle cell disease having experienced a clinical stroke between January 1, 2000, and September 30, 2009	initiated on HU every 2 weeks until max. tolerated dose achieved, then monthly for 3 months, and every 3 months thereafter; max. tolerated dose ~30 mg/kg/day; followed for 43 person years	One patient (10%) in the HU treated group had another stroke 75 weeks after initiation of treatment; patient was diagnosed with moya moya syndrome. Twenty of 33 patients (61%) of untreated group had another stroke; average time to recurrence was 55 weeks (3-377 weeks).	Ali et al. (2011) [PMID:21898530]
1F, 3.8 years old	sickle cell disease	initiated at 4 years of age to a maximal tolerated dose of 25 mg/kg	Transcranial Doppler studies showed that HU treatment was associated with normalization of the time average mean of the maximum velocity in the left middle cerebral artery and resolution of vascular stenosis. Development of neutropenia led to reduction of HU dosage to 17 mg/kg by 8 years of age.	Grace et al. (2010)

Abbreviations: bw= body weight; F = female(s); Hb = hemoglobin; HU = hydroxyurea; M = male(s); MN = micronuclei; n.p. = not provided

In patients with sickle cell disease, HU increased hemoglobin and fetal hemoglobin levels and average blood cell volume, while decreasing events/parameters such as the rate of vaso-occlusive crisis, acute chest syndrome, transfusions, polynuclear neutrophils, and platelets (Bradai et al., 2009 abstr.; Mellouli and Bejaoui, 2008 [PMID:18164913], Thornburg et al., 2009 [PMID:19061213]; Trelinski et al., 2009 [PMID:19741509]; Voskaridou et al., 2009 abstr.; Wang and Thompson, 2010 abstr.; Wang et al., 2011). Decreases in the number of transfusions were also reported in patients, including children, with thalassemia (Bradai et al., 2009 abstr.; Mtvarelidze et al., 2008 [PMID:18403819]; Zamani et al., 2009). More recently, HU was found to be an alternative therapy to treat secondary polycythemia caused by incurable cyantic congenital heart disease (CCHD) (Boussaada et al., 2007 [PMID:17657936]). It was also effective in the treatment of secondary erythrocytosis in CCHD. HU improved symptoms of hyperviscosity (fatigue, dyspnea, recurrent stroke, recurrent transient ischemic attack, and headaches) in patients while decreasing red blood cell count and increasing both mean corpuscular hemoglobin and volume (Reiss et al., 2007 [PMID:17506064]). HU chemotherapy was used to treat a 12-year-old boy for 18 months to prevent the occurrence of desmoid tumor (Ramirez et al., 2009 [PMID:19322113]). A recent case report described the use of HU to treat chronic immune thrombocytopenic purpura in a woman with sickle cell disease (Schloemer et al., 2011 lett.).

Adverse Effects

HU treatment is not without side effects. It induced cutaneous lesions typical of amyopatic dermatomyositis, usually months to years after the start of treatment. In most patients, improvement of clinical manifestations occurred following discontinuation of HU (Dourmishev and Dourmishev, 2008 [PMID:18613806]). Of 27 patients treated with HU, 89% presented with cutaneous or mucous side effects (e.g., cutaneous xerosis, actinic keratosis, pruritus, nail pigmentation, and cutaneous ulceration). Side effects were associated with a cumulative dose of 2 kg (Dumont-Wallon et al., 2006). In a separate study, 210 patients with a variety of diseases (essential thrombocythemia, chronic myelogenous leukemia, polycythemia vera, and chronic myeloproliferative syndromes) were monitored for the development of skin lesions. Of the patients examined, 30 developed skin-related side effects: melanonychia, acral ulcers, dermopathy, multiple cutaneous carcinomas, and diffuse melanosis (Aste et al., 2006). Out of 124 patients with essential thrombocythemia, polycythemia vera, and myelofibrosis, five women over the age of 75 developed leg ulcers (Ruzzon et al., 2006 [PMID:16804363]). Although HU is effective on patients with myeloproliferative disorders, adverse effects such as macrocytic anemia, fever, myelodysplasia, and leg ulcers have also been reported. In a retrospective cohort study of 152 patients with polycythemia vera and essential thrombocytosis, adverse effects occurred in 16 individuals. In most cases, the effects were minor compared to symptoms of the disorder and were reversed upon the cessation of HU therapy (Randi et al., 2005 [PMID:16011962]). Other adverse effects of HU treatment reported in some recent studies are presented in **Table 1**.

In children with sickle cell disease with avascular necrosis of the femoral head (AVN), the presence of AVN was significantly associated with HU use ($p = 0.05$) (Mahadeo et al., 2008 abstr.). HU was implicated as the causal drug for drug-induced hypersensitivity syndrome in one individual (Ben m'rad et al., 2009 [PMID:19440116]). Two case studies reported multiple cutaneous neoplasms, leg ulcers, multiple hypertrophic actinic keratoses, and squamous cell

carcinoma associated with HU treatment (Hoff et al., 2009 [PMID:19756436]; Wiechert et al., 2009 [PMID:19096810]). Of 136 patients with essential thromocythemia and undergoing HU treatment, six experienced hemorrhagic events and 26 experienced thrombotic events (Palandri et al. (2009 [PMID:19208420]). In a retrospective study, 11.2% of patients treated with HU developed a second malignancy (median time = 87 months) after diagnosis with essential thromocythemia; however, no association between treatment and an increased risk of the development of a second hematological malignancy was established (Radaelli et al., 2008 [PMID:18796244]).

Several case reports have also described adverse effects associated with HU treatment. For example, an elderly woman with polycythemia vera undergoing HU treatment developed pulmonary thrombovasculities and desquamation alveolitis and eventually died (Skipsky et al., 2009). In another case report involving an elderly woman, exposure to HU for 13 years was associated with development of cutaneous squamous dysplasia; evaluations of lesions indicated development of Bowenoid AK and Bowen disease (Schleussinger et al., 2011 lett.). A 76-year-old woman on long-term HU therapy for polycythemia vera developed a variety of skin abnormalities/lesions. Cutaneous lesions on her hand and finger progressed and led to the development of tumors, specifically basal cell carcinoma and poorly differentiated sarcomatoid squamous cell carcinoma (Radic et al., 2011 [PMID:21933645]). Three recent case reports have described the development of melanonychia associated with HU treatment. The effects were observed in patients with different medical histories (e.g., essential throm-bocythemia and polycythemia vera) and had been treated with HU for varying lengths of time (4 weeks – 11 years) (de Franca et al., 2011; Kluger et al., 2011; Su et al., 2011). Development of porokeratosis, which is manifested clinically as annular plaques with a peripheral keratotic rim, was described in two men with polycythemia rubra vera that were being treated with HU for ≥2 years (Kanitakis et al., 2011 lett.). HU-induced hyperkalemia was proposed in an 81-year old woman diagnosed with polycythemia vera; length of HU-treatment was not provided (Marusic et al., 2011 lett.).

Compared to these studies, a lack of effect was observed in a child that received an accidental overdose of HU. An 11-month-old infant with sickle cell anemia ingested 9800 mg (612 mg/kg) HU. Serum level of HU 4 hours after ingestion was ~7756 μM. While transient hematological effects were noted (e.g., reduction in leukocyte counts), no liver or renal effects were observed. No other effects were reported, and complete recovery of all effects was noted by 7 days after exposure (Miller et al., 2011 [PMID:21744485]).

Reproductive and Teratological Studies

Overall, studies conducted in individuals taking HU showed no adverse effects in parent reproductive parameters or in offspring. In two pregnant women who had been taking HU for treatment of sickle-β thalassemia (but discontinued upon discovering they were pregnant), no abnormal congenital malformations were observed in their babies. Over 30 pregnant women with sickle cell disease or with different myeloproliferative disorders exposed to HU in the first, second, or third trimester delivered healthy babies (Italia et al., 2010). Several additional studies reported no teratogenic or mutagenic effects in newborns of 19 women who had taken HU (Ballas et al., 2009). HU was even used in place of imatinib to treat a 36-year-old pregnant

patient with chronic myeloid leukemia. A healthy baby (i.e., no birth defects and normal blood counts) was delivered (Dolai et al., 2009 lett.).

Details of studies of reproductive and development toxicity are presented in **Table 1** and results are briefly summarized below.

In a 17-year follow-up of surviving patients enrolled in the original Multicenter Study of Hydroxyurea in Sickle Cell Anemia trial, no association between HU use, whether in female or male patients, and teratogenic effects or neonatal abnormalities was observed. For six women taking HU, the pregnancy outcome was the following: 2 live births (one full term and one premature), 3 elective abortions, and 1 spontaneous abortion. For the three outcomes that had "probable" HU usage (i.e., known usage at conception, sometime during gestation, and at the time of delivery), 2 were elective abortions and 1 spontaneous abortion. For 10 men taking HU, pregnancy outcomes of female partners were the following: 6 live births (4 full term, 1 premature, and 1 gestational age >37 weeks but weight unknown), 2 elective abortions, and 2 spontaneous abortions. Additionally, no delays in development milestones were observed. [Note: The authors commented that the conclusions are limited, since the reasons for the elective abortions are unknown] (Ballas et al., 2009).

Although studies have shown HU treatment to have effects on sperm parameters (Berthaut et al., 2008; Grigg, 2007 [PMID:17316339]; Lukusa et al., 2009 [PMID:19437321]), some parameters can be reversed. In one study, a 35-year-old male with sickle cell anemia receiving HU for three years had azoospermia but returned to normal spermatogenesis within 6 months after ceasing HU treatment. His wife conceived within 4 months after his stopping the treatment, giving birth to a healthy baby (Masood et al., 2007 [PMID:17333529]). Recovery of spermatogenesis was also seen in a patient being treated with HU for polycythemia rubra vera. After >10 years of treatment, he was azoospermic. Upon cessation of treatment, sperm count had returned to normal numbers within three months (Grigg, 2007 [PMID:17316339]).

Genotoxicity

In patients receiving HU for sickle cell disease, a significant increase in the number of micronuclei was observed in white cells and correlated with treatment length and dose (Maluf et al., 2009). Additionally, a positive association was found between DNA damage and the mean dose and a negative association between DNA damage and treatment length (Friedrisch et al., 2008 [PMID:17988936]). Patients with polycythemia vera developing into terminal acute myeloid leukemia or myelodysplastic syndrome had clonal chromosome abnormalities (Swolin et al., 2008 [PMID:18351338]). Epigenetic changes and alterations in miRNA expression in response to HU treatment have been recently described (Walker et al., 2011a [PMID:21921042]). Compared to these studies, a recent evaluation by McGann and colleagues (2011a [[PMID:21542824]) showed that HU treatment did not induce chromosomal damage formation in children treated for up to 12 years. In another study, no significant genotoxic effects (e.g., chromosome breaks, chromatid breaks, and illegitimate VDJ recombination event) were noted in infants (mean 13.6 months) treated with 20 mg/kg/day HU for two years when compared to control patients (McGann et al., 2011b [PMID:22012708]). Details of these studies are presented in **Table 1**.

In an older study, peripheral blood (97% pure malignant population) was obtained from a 60-year-old female patient with acute myelocytic leukemia before and after HU treatment (1.5 g/m^2). Within seven hours of treatment, a greater than fivefold increase in DNA methylation was noted (Nyce, 1989).

Other Data

Mutational status has been shown to be associated with the effects produced by HU. In a recent study by Panova-Noeva and colleagues (2011 [PMID:21750318]), the relationship between the presence of the *JAK2V617F* mutation and HU effect on platelet and immature platelet count, percentage of immature platelets, and percentage of highly fluorescent immature platelet fraction was evaluated. [ILS Note: the abbreviation H-IPF was not provided in the article, but a Google search indicated that it may refer to highly fluorescent immature platelet fraction.] A total of 46 essential thrombocythemia and 38 polycythemia vera patients were evaluated; 23essential thrombocythemia and 18 polycythemia vera patients taking HU (dosing not provided). In patients negative for the mutation, there was no difference between HU-treated and non-treated groups. Comparatively, in patients positive for the mutation, HU significantly decreased platelet and immature platelet count.

Clinical Trials

A total of 96 trials were identified at ClinicalTrials.gov (as of April 12, 2010) using the keyword "hydroxyurea." Of these, 76 were closed (i.e., no longer seeking new volunteers): 25 were active and not recruiting, 43 were completed, 7 were withdrawn or terminated, and 1 was recruiting by invitation only. Several studies were focused on children with sickle cell anemia. These studies evaluate the long-term effects of HU, its efficacy in treating sickle cell anemia, ability to prevent or reverse chronic organ damage, and the efficacy of HU in reducing strokes and treating secondary pulmonary hypertension (ClinicalTrials.gov, 2010). Results from unpublished clinical trials[1] with HU are presented in **Table 2**.

A study in 27 patients with types II and III spinal muscle atrophy was recently completed. In the study, patients with confirmed SMN1 gene deletion were assigned to either the placebo or treatment group for 6 months. Patients treated with HU initially consumed 7.5 mg HU/kg/day. Monthly increases of 2.5 mg/kg/day were given up to a maximum concentration of 15 mg/kg/day. Low absolute neutrophil count was 4% in the HU group compared to 2% in the placebo group. No significant hepatic or renal toxicity was noted. Motor unit number estimation was significantly higher in patients treated with HU compared to placebo patients. A trend toward increased scores in Gross Motor Function Measurement subscales was also noted in the HU group (Wang et al., 2008 abstr.).

[1] Studies included in the table involved use of only HU as therapy for which no final study reports were identified in a March 12 and 22, 2010, multi-database searches described in the search strategy or in April 25 or November 15, 2011, PubMed searches (**Appendix B**).

Table 2. Unpublished Clinical Trials with Hydroxyurea

Study Title and ClinicalTrials.gov Identifier*	Study Population, Design, Purpose, and Dates	Treatment	HU Adverse Effects Monitored	Principal Investigator/Affiliation
Long Term Effects of Erythrocyte Lysis, NCT00842621	- Pediatric participants (5-19 years) with severe sickle cell disease or other forms of sickle cell disease who are or are not receiving treatment and pediatric and adult patients with non-sickling hematological disorders. - Observational, prospective cohort study. - Study evidence of pulmonary hypertension and the relation of tricuspid regurgitation jet velocity and intravascular hemolysis, etc. - Feb. 2009-June 2014	HU or chronic transfusions to be followed for up to 5 years	Not given	R.E. Ware/St. Jude Children's Research Hospital
Phase I/II Randomized Study of Hydroxyurea With or Without Clotrimazole in Patients With Sickle Cell Anemia, NCT00004492	- Sickle cell disease patients ≥18 years of age who have received HU for ≥6 months at a stable dose of ≥5 mg/kg bw/day for ≥3 months. - Randomized study in two treatment arms: HU + clomatrizole (phase I) or HU alone (phase II) for 12 months. - Compare the efficacy of HU ± clomatrizole in limiting severity of anemia and hemolysis rate. - October 1999- [Note that this study is said to be currently recruiting participants and the description was last updated June 23, 2005.]	Phase II: Oral HU for 12 months	Not given	E.P. Orringer/University of North Carolina
A Trial of Hydroxyurea in Spinal Muscular Atrophy, NCT00485511	- 60 type II and III spinal muscular atrophy patients ≥4 years of age. - Single-center, randomized, double-blind, placebo-controlled, prospective trial. - Evaluate efficacy and safety. - June 2007-June 2009.	HU for 2 years	Safety monitoring done to investigate the adverse effects. A smaller study with 33 spinal muscular atrophy patients found that doses <30 mg/kg bw/day were safe (Liang et al., 2008 [PMID:18166199]).	Y.J. Jong/Kaohsiung Medical University Chung-Ho Memorial Hospital
Long-Term Effects of Hydroxyurea in Children With Sickle Cell Anemia (The BABY HUG Follow-up Study) (Official title: Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) Follow-up Study), NCT00890396	- 167 children 2-7 years of age who had participated in the Baby Hug study. - Multi-center (14) observational cohort study - Evaluate the long-term effects of HU in children who participated in the earlier study and determine the optimal age to begin treatment. - Sept. 2008-Dec. 2011	Patients' doctors may continue to treat them with HU.	Both risks and benefits will be assessed.	B. Thompson/Clinical Trials and Surveys Corporation

Study Title and ClinicalTrials.gov Identifier*	Study Population, Design, Purpose, and Dates	Treatment	HU Adverse Effects Monitored	Principal Investigator/Affiliation
Long Term Follow Up in Sickle Cell Patients Treated by Hydroxyurea, NCT00480974	- 20 sickle cell disease patients aged 5-40 years that were treated for 5-12 years with HU, "beginning in childhood." - Observational retrospective study (case-only). Non-probability sample. - Provide a long-term follow-up. - May 2007-Dec. 2008.	Patients treated with HU 5-12 years. Doses not given.	Not given	K. Ariel/HaEmek Pediatric Hematology Unit, Medical Center, Israel
Hydroxyurea for Children and Young Adults With Sickle Cell Disease and Pulmonary Hypertension (Official title: A Pilot Study of Hydroxyurea for the Treatment of Pulmonary Hypertension in Children and Young Adults with Sickle Cell Disease), NCT00350844	- Sickle cell disease patients aged 10-25 years with pulmonary hypertension who had not been treated with HU. - Non-randomized, uncontrolled, single-group assignment, open-label study. - Study safety and efficacy. - July 2006-estimated June 2008 (still recruiting patients; last update Feb. 2009).	HU. Doses and duration not given.	Standard screening for toxicity to be performed monthly.	R.I. Liem/Children's Memorial Hospital, Chicago, IL; J. Panepinto/Children's Hospital Wisconsin and Medical College of Wisconsin, Milwaukee, WI
Hydroxyurea in Treating Patients With Recurrent and/or Unresectable Meningioma, NCT00006119	- 60 patients ≥16 years of age (30 per stratum; stage I vs. II or III) with meningioma (brain and central nervous system tumors) and life expectancy of >3 months. - Multi-center phase II interventional treatment study - Determine effectiveness for this condition. - July 1999- (Said to be ongoing at last update on July 23, 2008.)	HU for 2 years	Toxicities of regimen monitored (presumably at 3-month intervals)	D. Frappaz/Centre Leon Berard (Four other French medical centers involved.)
Effect of Hydroxyurea as Treatment for Primary Desmoid Tumors, NCT00978146	- 10 patients aged ≤21 years with desmoid tumors/fibramatosis. - Phase II efficacy study with single-group assignment and open label. - Determine efficacy for treating the condition. - October 2009-January 2015 (No updates since Sept. 15, 2009, when the study was yet to open for patient recruitment.)	Starting HU dose 20 mg/kg bw/day. Therapy to continue ≤1 year as long as HU reduces or stabilizes tumor size and HU toxicities are manageable.	Examinations every 3 months. Data to be collected on adverse events. [Note: Abstract on preliminary results published in 2008 in a series of 14 pediatric patients stated that HU treatment was associated with complete and partial responses in 4 cases, tumor stabilization in 7 cases, and disease progression in 3 cases (Meazza et al., 2010 lett.).]	N.J. Balamuth (Responsible Party); R.B. Womer (Study Chair)/Children's Hospital of Philadelphia (PA)

Study Title and ClinicalTrials.gov Identifier*	Study Population, Design, Purpose, and Dates	Treatment	HU Adverse Effects Monitored	Principal Investigator/Affiliation
Stroke With Transfusions Changing to Hydroxyurea (SWiTCH), NCT00122980	- ~130 children aged 5.0-18.9 years who have sickle cell disease and had suffered a stroke with cerebral infarction after age 12 months and had been treated for ≥18 months by red cell transfusions. 65 subjects per treatment arm (half will be treated with HU; half will remain on transfusion and iron chelation) - Randomized, Phase III, open label. - Determine efficacy in preventing further strokes while avoiding iron overload. - Aug. 2005-July 2012	HU plus phlebotomy to reduce iron load	Growth and development at base line and at 30 months will be compared.	R.E. Ware/St. Jude's Research Hospital R.W. Helms/Rho Inc. [A publication related to this study did not report on HU treatment (Aygun et al., 2009 [PMID:19344396]).]
Inflammatory Response to Hydroxyurea Therapy in Sickle Cell Disease, NCT00784082	- 100 children >3 years of age and of sub-Saharan extraction with sickle cell disease. Subgroups: those with and without a history of vaso-occlusions, those with and without HU treatment, and a healthy control group. - Observational. - Determine if the induction of a natural anti-inflammatory response via the hypothalamus-pituitary-adrenal axis is a factor in the therapeutic activity of HU. - September 2009-June 2010 (Still recruiting as of last update on April 1, 2009.)	20-25 mg/kg bw/day "since at least 3 months"	Numerous inflammatory biomarkers such as cortisol and ACTH will be monitored.	M.-H. Odièvre/Assistance Publique - H
Evaluating the Safety of G-CSF Mobilization in Individuals With Beta Thalassemia Major, NCT00336362	- 24 patients aged 18-50 years with β-thalassemia, half of whom have been splenectomized. - Non-randomized, open label safety/efficacy study. - Investigate the safety and feasibility of collecting peripheral blood stem cells from patients with this condition, a necessary step before attempts at gene transfer. - July 2006-February 2010 (Last updated August 21, 2009, when recruitment was still ongoing.)	All patients treated with G-CSF (filgrastim) for several days to increase stem cell nos. Half pretreated with up to 25 mg HU/kg/day for 1 month to reduce clotting risk in splenectomized patients and decrease spleen size in non-splenectomized patients.	Safety of peripheral blood stem cells mobilization with G-CSF with or without HU pretreatment will be assessed by monitoring for study-related toxicity.	E. Yannaki/George Papanicolaou Hospital, Thessaloniki, Greece

Study Title and ClinicalTrials.gov Identifier*	Study Population, Design, Purpose, and Dates	Treatment	HU Adverse Effects Monitored	Principal Investigator/Affiliation
Hydroxyurea for the Treatment of Patients With Sickle Cell Anemia (Official title: Effect of hydroxyurea on fetal hemoglobin synthesis in patients with sickle cell anemia), NCT00001197	- 50 severely affected patients with homozygous sickle cell disease or other sickling disease who are >18 years of age with relatively well preserved renal and hepatic function (must be able to tolerate an extensive period without blood transfusion). - Phase II interventional treatment study. - Assess hematological changes that occur longitudinally. - February 1984-October 2009 (First received Nov. 3, 1999. Last updated March 26, 2010)	HU doses not given.	Will examine for unanticipated long-term side effects.	G.P. Rodgers/National Institute of Diabetes and Digestive and Kidney Diseases

*See Section 12.0 for URLs to each study. Source: ClinicalTrials.gov, a service of the U.S. National Institutes of Health (http://www.clinicaltrials.gov/ct2/search; last accessed on April 7, 2010). Selected studies involved ise of only HU as therapy for which no final study reports were identified in a March 12 and 22, 2010, multi-database searches described in the search strategy or in April 25 or November 15, 2011, PubMed searches (**Appendix B**).

Abbreviations: bw = body weight; HU = hydroxyurea

Chemical Disposition, Metabolism, and Toxicokinetics

HU clearance is primarily via the kidneys. In sickle cell anemic adults with normal renal function, intake of a commercially available HU capsule (15 mg/kg) had a maximal concentration (C_{max}) of 28.32±11.0 µg/mL, area under the curve (AUC) of 81.66±15.5 µg·hr/mL, and half-life ($T_{1/2}$) of 3.14±0.9 hours (Rogers et al., 2005 abstr.).

According to a phase 1 pharmacokinetics study, HU tablets (1000 mg) and capsules (500 mg) caused no significant differences in several parameters when measured in children and adults with sickle cell disease (see **Table 3**). Children (n=11, ages 4-19 years) were given HU tablets (mean dose: 21.4 mg/kg/day) for 14 days. Adults (n=15, ages 21-49 years) were in a randomized crossover study comparing tablets to capsules (mean dose: 20.8 mg/kg/day [capsules]) for 8 days each with a 2- to 4-week break. The difference in the renal excretion between children and adults was suggested to be due to the increase of glomerular filtration rate in adults (De Montalembert et al., 2005 abstr.).

Table 3. Pharmacokinetic Parameters (Mean Values) in Sickle Cell Disease Children and Adults[1]

Study Group	C_{max} (mg/L)	C_{min} (mg/L)	T_{max} (hr)	AUC_{0-24} (mg/hr/L)	$AUC_{0-\infty}$ (mg/hr/L)	$T_{1/2e1}$ (hr)	% Renal Excretion
Children	29.61	1.09	0.75	124.16	142.20	7.49	36.90[2]
Adults (tablets)	29.47	0.85	1.19	135.40	143.62	6.27	58.32
Adults (capsules)	27.62	0.78	1.34	138.41	145.91	5.69	60.21

[1]Copied from publication with minor revisions.
[2]Value is similar to the value reported in adults with cancer.

In a study in which younger children with sickle cell anemia (n=22, mean age = 14.5 months [BABY HUG clinical trial]) were orally administered HU (20 mg/kg), first-dose pharmacokinetic parameters were 19.81±5.8 µg/mL for C_{max}, 68.82±11.5 µg hr/mL for AUC, and 2.36±0.99 hours for $T_{1/2}$, which were lower than those of sickle cell anemic adults. In addition, infants ≤15 months were observed to have a shorter $T_{1/2}$ (2.1 versus 2.8 hours) and lower 8-hour predicted measureable HU concentration (1.2 versus 2.1 µg/mL) than children aged 16-18 months. No differences in C_{max} or AUC were seen (Rogers et al., 2005 abstr.). In another study, pediatric patients (n=40; ages not provided [HUSTLE clinical trial]) were administered HU (20 mg/kg). The apparent oral clearance of HU was 0.252±0.080 L/hr/kg, slightly higher than that published for adults, and the median peak time was 0.55 hours (0.467-2.2 hours). Significant pharmacokinetic interindividual variability was seen (30.3% for C_{max}, 26.0% for $T_{1/2}$, 46.7% for apparent oral clearance [mainly related to weight], and 61.6% for apparent volume of distribution). In addition, when the patients were grouped on the basis of time to peak concentration, rapid and slow absorption was observed. In children with rapid absorption, the predicted median time to peak concentration, based on a linear one-compartment model, was 18 minutes. They also had higher median C_{max} (74% more) and median AUC (33% more) than those with slower absorption (Ware et al., 2008 abstr.). Both studies showed no contribution from glomerular filtration rate.

A more recent study yielded similar results to those described in the abstract noted above. In a group of 87 patients given 20 mg/kg HU, the mean C_{max} was 26.13 µg/mL with a half-life of

1.70 hours. The time to the maximal concentration was 0.82 hours, overall. As noted previously, there was significant interindividual variability (65% could be accounted for by weight) and patients could be differentiated as having a "fast" or "slow" absorption profile. A majority of the patients (51 of 87) were identified as having "fast" absorption (C_{max} at 15 or 30 minutes), while the remained had "slow" absorption (Cmax at 60 or 120 minutes). When the two absorption profiles were compared, the fast absorbers has significantly higher C_{max} and lower mean residence time (Ware et al. 2011 [PMID:21876119]).

In a nine-year-old female administered HU (500 mg) [no additional information regarding health status of individual provided.], HU plasma levels were evaluated for four hours post administration. A maximal plasma concentration of HU was achieved approximately one hour after administration (30-35 mg/L). Plasma concentration decreased in a time-dependent manner until the end of the study (Kettani et al., 2009 [PMID:19144580]).

Population pharmacokinetic and pharmacodynamic models were developed based on data from clinical studies using adults that were receiving HU to assess the exposure-efficacy relationships and their variability. The models were also used to assess two different dosing regimens. The developed models were two-compartment models with first-order absorption and elimination. Based on the model, the pharmacokinetics was estimated to be linear. Additionally, it was proposed that the variability in response to HU was associated, in part, to the pharmacokinetics and pharmacodynamics of the chemical. Studies suggested that the steady-state value of the mean corpuscular volume at 3 months was not predictive of the fetal hemoglobin percentage value at 26 months, further suggesting that the fetal hemoglobin value was a more appropriate biomarker for monintoring HU treatment. Simulations using the model showed that continuous HU dosing led to a stronger response than intermittent HU dosing (Paule et al., 2011).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

HU is distributed throughout the body. In mice, urea was identified as the main metabolite in urine. The elimination half-life was less than 0.5 hour in rodents (CERHR, 2008).

Male Sprague-Dawley rats were injected 0.5 mL radiolabeled HU as a bolus dose or 0.5 radiolabeled HU followed by short infusions (total volume = 1.9 mL) through a catheter in the tail vein. Brain uptake of radiolabeled HU was measured using positron emission tomography (PET) and whole blood and plasma radioactivity levels were measured in a well-counter that was cross-calibrated with the PET system. Results showed that overall radiolabel levels were lower in the brain than compared to plasma levels. Calculated distribution and elimination half-lives were 1.7 and 171 minutes, respectively. The brain-to-plasma ratio at 60 minutes post-administration ranged from 0.13 to 0.18 (Syvanen et al., 2007 [PMID:19356042]).

HU (10-300 μM) was not metabolized *in vitro* when incubated with human liver microsomes in the presence or absence of nicotinamide adenine dinucleotide phosphate (NADPH), suggesting that cytochrome P450 was not involved in HU metabolism at this concentration range. Additionally, studies suggested that HU was not a substrate for P-glycoprotein *in vitro* (Sassi et al., 2010 [PMID:19817872]). Comparatively, *in vitro* incubation of HU (1 mM) with rat liver homogenates increased nitrate and nitrite formation. Addition of NADPH decreased nitrate and nitrite formation. Metabolism was time- and concentration-dependent. Similar to *in vitro* human

studies, HU metabolism was not observed in the presence or absence of NADPH when incubated with rat liver microsomes (Huang et al., 2006 [PMID:16632127]).

In vitro studies with an artificial bilayer indicated that passive diffusion of HU was limited. Additional studies showed that HU was a substrate for a variety of solute carrier transporters, specifically OATP1A2, OATP1B1, OATP1B3, OCTN1, and OCTN2, and urea transporters A and B (Walker et al., 2011b [PMID:21256917]).

9.1.3 Acute Exposure

Acute toxicity values for HU are presented in **Table 4**.

Table 4. Acute Toxicity Values for Hydroxyurea

Route	Species (sex and strain)	LD_{50}
i.p.	Mouse (sex and strain n.p.)	5800 mg/kg
i.v.	Mouse (sex and strain n.p.)	2350 mg/kg
oral	Mouse (sex and strain n.p.)	7330 mg/kg
i.p.	Rat (sex and strain n.p.)	>4700 mg/kg
i.v.	Rat (sex and strain n.p.)	4730 mg/kg
oral	Rat (sex and strain n.p.)	5760 mg/kg

Source: ChemIDplus (undated)
Abbreviations: i.p. = intraperitoneal; i.v. = intravenous; LD_{50} = lethal dose for 50% of test animals; n.p. = not provided

9.1.4 Short-Term and Subchronic Exposure

Short-term and subchronic effects of HU were obtained from drug labels. Overall, doses that exceeded clinical levels produced cardiovascular and hematological adverse effects in some laboratory animals [species not provided]. Bone marrow hypoplasia, pulmonary congestion, and mottling of lungs were noted in rats. At higher doses, hepatic cell damage with fatty metamorphosis was noted in rats treated with doses >1200 mg/kg/day for ≥37 days (CERHR, 2008).

9.1.5 Chronic Exposure

No data were located.

9.1.6 Synergistic/Antagonistic Effects

Synergism/Antagonism of HU Effects

The protective effects of the cyanobacterium *Spirulina maxima* (SP) against HU-induced teratogenesis were evaluated in CD-1 mice. Females were given SP or its extract (SPE) via intragastric administration at doses ranging from 100-1000 and 100-400 mg/kg, respectively, on gestation days 1-9. On gestation day 9, mice were given an i.p. dose of HU (300 mg/kg), one hour after SP or SPE administration. Mice were killed on gestation day 11 and fetuses were examined for developmental abnormalities. In dams treated with HU alone, there were 78 viable embryos from a total of 152. Of these 78, 53 were identified as having developmental abnormalities (e.g., open neural tube and retarded hind and forelimb buds growth). Administration of SP or SPE significantly increased the number of viable embryos at all doses tested and decreased the number of embryos with developmental abnormalities at ≥500 mg/kg and ≥100 mg/kg, respectively. The antioxidant effects of SP and SPE (as assessed by

quantification of thiobarbituric acid reactive species) was proposed to be related to the protective effects noted (Vazquez-Sanchez et al., 2009 [PMID:19703510]).

Dimethyl sulfoxide (DMSO) was also shown to protect embryos from the teratogenic effects of HU. *In vitro* exposure of early somite mouse embryos showed that DMSO did not produce tertogenic effects while HU increased formation of developmental abnormalities (e.g., neural closure tube defects). Co-treatment with DMSO and HU reduced the incidence of developmental abnormalities and increased embryonic viability (Perez-Pasten et al., 2006 abstr.).

Calcium channel blockers diltiazem (100 μM) or verapamil (100 μM) enhanced cell growth inhibition induced by HU (5-50 μM) in a human intraosseous malignant meningioma cell line. At higher HU concentrations (100-200 μM), the calcium channel blockers did not enhance cell growth inhibition when compared to HU treatment alone. A similar effect was noted in a primary cell culture prepared from a human sphenoid wing benign meningioma. [Note: Compared to studies in the cell line, the effect of increasing concentrations of HU alone on cell growth was not graphically provided.] *In vivo* studies, using the mouse flank xenograft model, yielded similar results; addition of the calcium channel blockers enhanced decreased tumor volume. [Note: Animals implanted with malignant meningioma and treated with HU only were terminated on day 36 while other animals were terminated on day 56. Therefore, an exact comparison of the additive or synergistic effect of calcium channel blockers on HU effects is incomplete] (Ragel et al., 2006 [PMID:17143245]).

Synergism/Antagonism of Other Effects by HU

Co-exposure of K562 cells, a human chronic myeloid leukemia cell line, to celecoxib (40 μM) and HU (10 mM) downregulated cyclooxygenase-2 mRNA levels, produced significant growth inhibition, and increased apoptosis. The observed effects for co-incubation were greater than the effects observed when either chemical was administered separately (Zhang et al., 2006). In another study in the same cell line, the combination of HU and imatnib mesylate decreased cell viability, while the individual compounds did not decrease cell survival (Giallongo et al., 2011 [PMID:21198861]).

In HL-60 and T24 cells, HU blocked the azacitidine and decitabine-mediated inhibition of DNA methylation in a dose-dependent manner. At a dose of >0.5 mmol/L, HU fully blocked the effects of azacitidine in HL-60 cells. Inhibition of decitabine effects occurred at doses >0.1 mmol HU/L. This antagonistic effect only was observed when cells were exposed to the chemicals concomitantly. When cells were exposed to the chemicals sequentially, the observed HU anatagonistic was abolished (Choi et al., 2007).

HU also antagonized estradiol induced antagonism of Fas ligand (FasL) induced apoptosis in cultured bovine granulose cells. In the absence of other chemicals, HU had no effect on FasL-induced apoptosis while estradiol antagonized the observed effect. When the two agents were combined, the level of apoptosis was similar to that observed in control and HU-treated cells (Quirk et al., 2006).

In OUN-1 cells, HU and valproic acid upregulated expression in two natural killer-G2D ligands: MICA/B and ULPB2. The upregulation was greater than observed with either compound

separately (Lu et al., 2010 [PMID:20028385]). [ILS Note: It is unclear if the combination of compounds produced an additive or synergistic effect.]

9.1.7 Cytotoxicity

HU inhibits ribonucelotide reductase, which converts ribonucleotides to deoxyribonucleotides, and leads to inhibition of DNA synthesis. This results in S-phase cytotoxicity. *In vivo* studies have also demonstrated that HU is cytotoxic after intraperitoneal (i.p.) injection (CERHR, 2008).

In ReNcell CX cells, immortalized human neural progenitor cells, HU decreased BrdU incorporation and cellular viability. After incubation with HU for 4 or 48 hours, HU significantly inhibited BrdU incorporation at concentrations >1μM; BrdU incorporation was significantly inhibited at 100 μM after 24 hour incubation period. Comparatively, cellular viability was only decreased at 100 μM after the 24 and 48 hour incubation periods (Breier et al., 2008).

HU (1 and 2 mM) significantly increased the apoptotic index in HUVEC cells. Comparatively, addition of laminar shear stress inhibited HU-induced apoptosis. Proliferative activity was not affected by addition of 1 mM HU to cells (Zeng et al., 2011 [PMID:21791174]). [ILS Note: Authors noted that shear stress also proposed endothelial cell migration induced by HU.]

9.2 Reproductive and Teratological Effects

HU is a developmental toxicant in rats. It was noted that there was not sufficient data to evaluate the developmental effects of HU on immature animals or the postnatal effects of HU exposure. Limited reproductive studies suggest that HU impairs spermatogenesis in rats and mice; no reproductive studies in female animals were available (CERHR, 2008).

Four recent studies, three in Chinese, support previous studies on HU effects on male reproduction. A recent study in male Wistar rats showed that i.p. injection of 400 mg HU/kg significantly decreased Bcl-2 expression in testis 6 or 12 hours after treatment. Comparatively, HU treatment increased expression of Fas and Fast in testes (Zhou et al., 2009). In a separate study, HU administration by i.p. injection for five days increased the formation of DNA lesions in testicular cells (Yang et al., 2009). In another study, the sperm chromatin structure assay was used to assess HU effects on sperm nucleus DNA. Male Wistar rats injected with HU (100-400 mg/kg) showed evidence of DNA damage in a dose-dependent manner (Zhou et al., 2008). *In vitro* studies showed that HU exposure (96 hours) to rat testicular Sertoli-germ cell co-culture produced cytotoxic changes at concentrations ≥10 μM. Cytotoxic effects were also time-dependent (Liao et al., 2006 abstr.).

A study in a transgenic sickle cell disease model also supported the conclusion that HU produces adverse effects on male reproduction. Studies were conducted in transgenic sickle cell mice (TSCM) which express pathologies observed in sickle cell patients (e.g., sickle shaped red blood cells, elevated white blood cell count). Mice were treated with 25 mg HU for 28 or 56 days via oral gavage. While body weights were not affected by HU treatment, testis weight was decreased 40% and 64% after 28 and 56 days of treatment, respectively. After 56 days of treatment, testis dimensions were decreased 52%, and seminiferous tubule atrophy and Leydig cell prominence were noted. HU treatment also significantly decreased epididymal weight

(25%), sperm motility, and sperm density after 56 days of treatment. Significant decreases in plasma testosterone levels were noted after 28 and 56 days of treatment (Jones et al., 2009).

Recent studies also supported previous conclusions that HU effects embryonic development. Midbrain cells were harvested from 13-day-old rat embryos. Cells were cultured and then exposed to varying doses of HU for 5 days [doses not provided]. HU significantly inhibited proliferation and differentiation in a dose-dependent manner (Zhou et al., 2007). Pregnant Wistar rats were i.p. injected with 250 to 550 mg HU/kg on gestation day 11 and fetuses were evaluated on gestation day 21. Results showed numerous skeletal variations (structural changes that occur within the normal population) and malformations (changes that are likely to affect survival or development). The no observed effect level (NOEL) for observed variations was <250 mg/kg, while the NOEL for observed malformations (absent tympanic bone) was 250 mg/kg (Chahoud and Paumgartten, 2009 [PMID:19682677]).

An *in vitro* study in murine-derived stem cells (D3 cells), HU inhibited differentiation of cells into contractile cardiomyocytes in a concentration-dependent manner; inhibition of differentiation is correlated with embryotoxic potency. The EC_{50} of inhibition was 31.43 µM. HU also decreased cell viability; results suggested that decreased cellular viability was not full responsible for the observed effects on differentiation (Peters et al., 2008).

Oral administration of HU (30 mg/kg) for 28 days to female C57BL/6J significantly decreased ovarian weight, serum estradiol concentrations, and ovulation rate when compared to control mice. *In vitro* studies showed that continuous treatment of 2-cell embryos with HU decreased development to blastocyst stage. Intermittent HU treatment also decreased development to the blastocyst stage, but to a lesser extent than observed with continuous treatment (Sampson et al., 2010).

9.3 Carcinogenicity

No new carcinogenicity studies were identified. One study showed that HU increased the incidence of mammary tumors in rats after i.p. injection with 125–250 mg/kg three times per week for six months. Comparatively, in a study reviewed by the International Agency for Research on Cancer (IARC), pulmonary tumor formation was not affected by i.p. administration of increasing doses of HU for up to one year (CERHR, 2008). In 2000, the IARC stated that "Hydroxyurea is not classifiable as to its carcinogenicity to humans (Group 3)" (IARC, 2000).

9.4 Initiation/Promotion Studies

No data were located.

9.5 Genotoxicity

No new genotoxicity studies were identified. HU is mutagenic *in vitro* and *in vivo*. *In vitro* mutagenic studies have been done in bacteria, fungi, protozoa, and mammalian cells [strain and/or species not provided]. Clastogenic effects have been noted *in vitro* in hamster cells and human lymphoblasts and *in vivo* in rodents (Bristol Myers Squibb Co., 2007; CERHR, 2008).

HU also produced epigenetic effects in tumor cells. HTB-54 lung human epidermoid carcinoma cells were incubated with 10-100 µM HU for 24 hours or 0.5-5 µM HU for 48 hours. During the

24 hour incubation period, increased DNA hypermethylation was associated with decreased DNA synthesis and cell viability. At the highest concentration tested, hypermethylation was >150% of control values. Hypermethylation appeared to occur when DNA synthesis was inhibited ~90%. Increased DNA hypermethylation was also observed at lower concentrations when the incubation period was increased (Nyce, 1989).

HU inconsistently increased tail moment in V79 Chinese hamster cells at doses ranging from 50 to 500 μM after 18 hours of incubation. At the same concentrations and incubation time, HU increased chromosomal aberration formation; significant increases were noted at concentrations ≥100 μM. Chromatid breaks and exchanges were observed. Cytotoxicity was also noted; cell growth was reduced ~30% at 500 μM after 18 hours of incubation (Speit and Schutz, 2008 [PMID:18634899]).

HU increased micronuclei formation in a Chinese hamster lung fibroblast at doses of 3.1-25 μg/mL and in thymidine kinase$^{+/-}$ human lymphoblastoid TK6 cell lines at 10 and 2500 μg/mL (Hashimoto et al., 2009 [PMID:19747535]; Le Fevre et al., 2007 [PMID:17374387]). When based on size, the greatest proportion of micronuclei were <1/4 the diameter of the main nuclei (Hashimoto et al., 2009 [PMID:19747535]). HU also induced formation of micronucleated reticulocytes in Swiss albino mice at doses ranging from 12.5 to 200 mg/kg (dos Santos et al., 2011).

HU treatment significantly increased copy number variants (CNV) in culture normal human fibroblasts at concentrations ranging from 100 to 300 μM; deletions and duplications were both observed. [ILS Note: A U-shaped curve was noted.] Additionally, HU increased cell-doubling time. Analysis indicated that the increased CNV occur throughout the genome (Arlt et al., 2011 [PMID:21987784]).

Fibroblasts and lymphoblasts obtained from patients with spinal muscular atrophy were treated with HU for 24 and 48 hours. Treatment with HU caused the greatest increase on FL-*SMN* transcripts at 48 hours. According to FL-*SMN* mRNA and SMN protein in fibroblasts, there were two responders for HU. Lymphoblasts treated with HU showed an increase in FL/Δ7 ratios. After 48 hours, viability of spinal muscular atrophy cells was decreased by 15-20% in fibroblasts and by 20-30% in lymphoblasts with 1 mM HU. A one order of magnitude dose increment caused cellular viability decreases of 20% and 40%, respectively (Also-Rallo et al., 2011 [PMID:21610752]).

9.6 Cogenotoxicity

No data were located.

9.7 Immunotoxicity

No immunotoxicity data were located. However, a recent microarray study showed that HU treatment of a human vascular endothelial cell line upregulated pro-inflammatory cytokines including RANTES, monocyte chemotactic protein (MCP)-1, MCP-2, macrophage inflammatory protein-3a, interleukin (IL)-1a, IL-1b, IL-6, and IL-8. Genes were upregulated 2- to 30-fold. Comparatively, transcription of IL-4 and IL-10 were not altered by HU treatment. A similar upregulation of gene expression was also noted in primary cultures of human umbilical vein

endothelial cells (macrocirculation) and human pulmonary microcirculation endothelial cells (Laurance et al., 2008 abstr.).

9.8 Other Data

Vascular Effects

HU has been associated with acral erythema and leukocytoclastic vasculitis (Shahab et al., 2006 [PMID:16473650]). In human endothelial cell lines TrHBMEC and EA-hy 926, HU significantly decreased the release of the vasoconstrictor peptide endothelin-1 up to threefold through decreased gene expression. Expression of vascular cell adhesion molecule was also downregulated. Comparatively, expression of membrane-bound intercellular cell adhesion molecule 1 as well as the soluble form was upregulated by HU (Brun et al., 2003; Laurance et al., 2008 abstr.).

Mechanisms of Action

Proposed mechanisms of action associated with the therapeutic effects toward sickle cell anemia include upregulation of fetal hemoglobin synthesis; reduction in neutrophils, monocytes, and reticulocytes; effects on the sickle cell membrane, adherence molecule expression, or vascular reactivity; erythrocyte cation transport; nitric oxide formation; erythropoietin production; and/or red blood cell deformability (Steinberg, 2008 [PMID:19112541]). A recent study showed that HU produced genome-wide effects on transcription in mouse embryonic stem cells (Cui et al., 2010).

HU treatment in a sickle cell anemic patient caused upregulation of transcriptional and translational regulatory genes (e.g., EGR-1). The authors proposed that these genes played a role in the therapeutic effects associated with sickle cell anemia (Costa et al., 2007).

10.0 Structure-Activity Relationships

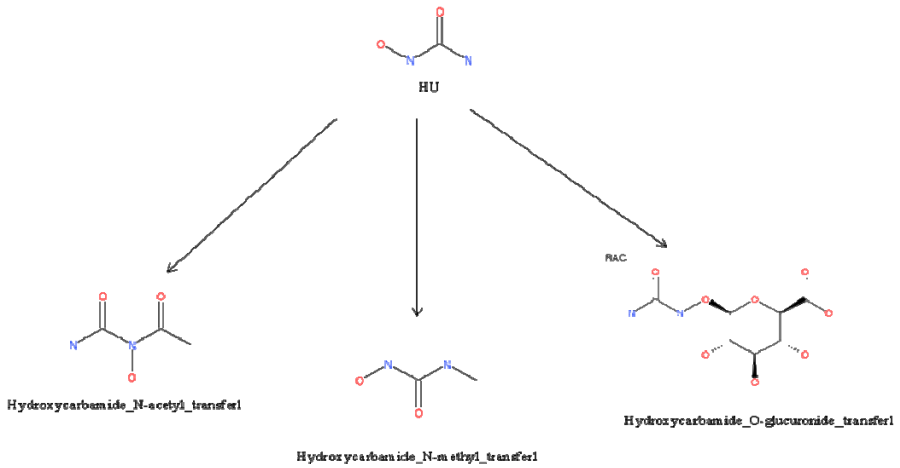
GeneGo Analysis (Appendix C)

For each GeneGo quantitative-structure activity relationship (QSAR) model, a QSAR value was calculated. For non-binary models, the calculated values ranged between two threshold values to be classified as active in the model. These threshold values corresponded to the negative logarithm of the activity for the most active compound in the training set and the negative logarithm of 50 μM (-1.7). For binary models (e.g., AMES mutagenicity binary model), the definition of an active chemical is model dependent. In addition to the QSAR value a Tanimoto similarity percentages (TP) was calculated which indicates the similarity percentage of HU to the most-similar compound in the training set. A detailed report with GeneGo results is provided in Appendix C. A summary of the results is provided below.

Overall, the evaluated QSAR models predicted carcinogenic and hepatotoxic effects. Based on the predicted targets, HU is proposed to effect one-carbon metabolic process and deoxyribonucleotide biosynthetic and metabolic processes.

Absorption, Distribution, Metabolism, and Excretion (ADME) QSARs

Three metabolites, identified as first-pass conjugated metabolites, were predicted. The metabolites were acylated, methylated, or glucuronidated conjugates of HU (see Figure below).



The CYP450 models, overall, predicted that HU would have some affinity for many of the evaluated isozymes (CYP1A2, CYP2B6, CYP2C19, and CYP2D6). The highest affinity was noted for CYP2C19 (pKm = 1.33). However, the TP values were <20% for all the models. Models that evaluated the inhibitory activity of HU and its metabolites yielded similar results. Compounds were identified as potential inhibitors CYP2D6 and CYP3A4; however, TP values were <20%. The one exception was the model for inhibition of human soluble epoxide hydrolase which predicted low inhibition activity by HU. Models of the Phase 2 metabolism enzymes sulfotransferases and UDP-glycosyltranferases predicted that HU would have affinity for the human sulfotransferase 1A1. However, as mentioned for the CYP models, the TP value was low (17.39). *In vitro* metabolism studies suggest that CYP enzymes are not involved in HU metabolism in rodents or humans. In rodents, the major identified metabolite was urea.

Additional ADME models evaluated blood-brain barrier penetration, binding affinity to human serum albumin, serum protein binding, water solubility, and octanol-water distribution coefficient of HU. The models predicted that HU could enter the brain and has lower protein binding potential to human serum albumin; the TP values for all predicted effects were <25%. The acetylated and methylated HU metabolites were also predicted to have some brain-penetrating ability. The prediction that HU could enter the brain is supported by *in vivo* studies.

Protein Binding QSAR for HU

While many of the protein binding QSAR models indicated that HU was considered active in the model, the TP values were typically <20%.

Therapeutic Activity QSARs for HU

Of the 25 models evaluated, one predicted that HU would be active (calculated value >0.5) and the TP value was >50%; anticancer activity was predicted for HU. This prediction supports the extensive literature of the anticancer effects of HU.

Toxic Effects QSARs for HU

Numerous toxic effects (calculated value >0.5 and TP value >50%) were predicted for HU. HU was predicted to be carcinogenic in mice and rats (1, TP=38.89-53.33) and hepatotoxic (0.73, TP = 52.94). HU was predicted to have mutagenic potential in the AMES mutagenicity binary

model (0.6 [1 defined as mutagenic], TP = 100). In the general cytotoxicity model for log growth inhibition in MCF7 model, where log GI_{50} from 6 to 8 is defined as toxic and values less than 3 are less toxic, a predicted value of 4.65 was determined (TP = 100). The model for general toxicity, based on log Maximum Recommended Therapeutic dose (mg/kg bw/day), yielded a value of 0.82 (TP = 52.94); chemicals above the 0.5 cutoff value are classified as less toxic.

There are conflicting studies on the carcinogenicity of HU One study showed that HU increased the incidence of mammary tumors in rats while another showed that pulmonary tumor formation was not affected by i.p. administration of increasing doses of HU for up to one year. Studies have also shown that HU is cytotoxic to a variety of cell types.

Possible Targets for HU and Metabolites

Based on structural similarity (≥75%), HU was identified as a potential inhibitor of several enzymes. Most of the identified targets were based on literature information regarding the activity of HU itself. Several members of the carbonic anhydrase family were previously shown to be HU targets, including carbonic anhydrase II, carbonic anhydrase IV, carbonic anhydrase I, carbonic anhydrase, and carbonic hydrase VA (Santos et al., 2007 [PMID:17251018]; Scozzafava and Supuran, 2003 [PMID:12713833]; Temperini et al., 2006 [PMID:16759856]). These enzymes are involved in the reversible hydration of carbon dioxide. As is known from previous studies, HU also inhibits the ribonucleotide reductase enzyme. *In vitro* studies indicate that HU inhibits ribonucleotide reductase activity low affinity; ID50 values ranged from 110 to 500 μM (Parker et al., 1977 [PMID:926124]; Sigmond et al., 2007 [PMID:17324380]; van't Reit et al., 1979 [PMID:458812]). No targets for the predicted metabolites were provided.

11.0 Online Databases and Secondary References Searched

11.1 Online Databases

National Library of Medicine Databases

PubMed

ChemIDplus – chemical information database that provides links to other databases such as CCRIS, DART, GENE-TOX, HSDB, IRIS, and TRI. A full list of databases and resources searched are available at http://www.nlm.nih.gov/databases/.

STN International Files

AGRICOLA	IPA
BIOSIS	MEDLINE
BIOTECHNO	PASCAL
CABA	Registry
EMBASE	TOXCENTER
ESBIOBASE	

Information on the content, sources, file data, and producer of each of the searched STN International Files is available at http://www.cas.org/support/stngen/dbss/index.html.

Government Printing Office

Code of Federal Regulations (CFR)

11.2 Secondary References

Budavari, S., Ed. 1996. Hydroxyurea. Monograph No. 4896. *The Merck Index*, 12th ed. Merck and Company, Inc., Whitehouse Station, NJ, p. 833.

12.0 References

URLs for Clinical Trials Listed in Table 2 (in order of appearance)
NCT00842621: http://www.clinicaltrials.gov/ct2/show/NCT00842621?term=hydroxyurea&rank=92
NCT00004492: http://www.clinicaltrials.gov/ct2/show/NCT00004492?term=hydroxyurea&rank=64
NCT00485511: http://www.clinicaltrials.gov/ct2/show/NCT00485511?term=hydroxyurea&rank=50
NCT00890396: http://www.clinicaltrials.gov/ct2/show/NCT00890396?term=hydroxyurea&rank=49
NCT00480974: http://www.clinicaltrials.gov/ct2/show/NCT00480974?term=hydroxyurea&rank=44
NCT00350844: http://www.clinicaltrials.gov/ct2/show/NCT00350844?term=hydroxyurea&rank=43
NCT00006119: http://www.clinicaltrials.gov/ct2/show/NCT00006119?term=hydroxyurea&rank=40
NCT00978146: http://www.clinicaltrials.gov/ct2/show/NCT00978146?term=hydroxyurea&rank=20
NCT00122980: http://www.clinicaltrials.gov/ct2/show/NCT00122980?term=hydroxyurea&rank=19
NCT00784082: http://www.clinicaltrials.gov/ct2/show/NCT00784082?term=hydroxyurea&rank=8
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Appendix A: Units and Abbreviations

ºC = degrees Celsius
μM = micromolar
bw = body weight
ADME = absorption, distribution, metabolism, and excretion
AVN = avascular necrosis of the femoral head
CCHD = cyantic congenital heart disease
CL/F = apparent oral clearance
CNV = copy number variants
DMSO = dimethyl sulfoxide
EC_{50} = concentration at which 50% of the maximum effect was induced
FasL = Fas ligand
g = gram(s)
g/cm^3 = gram(s) per cubic centimeter
FDA = U.S. Food and Drug Administration
GC/MS = gas chromatography/mass spectrometry
HPLC = high performance liquid chromatography
HSDB = Hazardous Substances Data Bank
HU = hydroxyurea
IARC = International Agency for Research on Cancer
IL = interleukin
i.p. = intraperitoneal(ly)
kg = kilogram(s)
L = liter(s)
MCP = monocyte chemotactic protein
mg = milligram(s)
mg/kg = milligram(s) per kilogram
mg/L = milligram(s) per liter
mm Hg = millimeter(s) of mercury
mM = millimolar
n.p. = not provided
NADPH = nicotinamide adenine dinucleotide phosphate
NOEL = no observed effect level
NTP = National Toxicology Program
PET = positron emission tomography
PMID = PubMed identification
QSAR = quantitative-structure activity relationship
SP = *Spirulina maxima*
SPE = *Spirulina maxima* extract
TP = Tanimoto similarity percentages
TSCM = transgenic sickle cell mice
UV = ultraviolet

Appendix B: Description of Search Strategy and Results

STN International files MEDLINE, AGRICOLA, CABA, IPA, BIOSIS, TOXCENTER, EMBASE, ESBIOBASE, BIOTECHNO, and PASCAL were searched simultaneously on March 12 and March 22, 2010. To simplify the description of the strategy, the second session has been edited so that answer numbers in the March 22 session correspond to those that had been used in the March 12 session and those that would have been assigned to subsequent answer sets if the sessions had all been conducted on the same day. The total numbers of answers in the reused answer sets increased slightly during the 10-day interval, so the March 22 date is noted. The answer sets corresponded to clinical trials (L13), reproductive toxicity (L28 and L32), and the remainder minus patents (L37). The numbers of full records downloaded were L13, 150; L28, 160; L32, 5; and L37, 106. Selections from L37 were primarily on pilot studies for clinical trials, other multipatient studies, adverse effects, and genotoxicity. Kinds of information that were already covered or were case reports of well-known adverse effects (e.g., pigmented nails and skin ulcers) were omitted. For the first clinical-trials answer set (L13), it was difficult to understand why hydroxyurea was mentioned when the title stated that the trial used a different drug. Perhaps some of these included a group treated with hydroxyurea too. Others might have mentioned that the drug tested was used for hydroxyurea-resistant patients.

```
L1        53835 S HYDROXYUREA OR HYDROXYCARBAMIDE OR HYDROXYL(W)UREA OR HYDROXYUREA
                OR 127-07-1
L2            7 S BIOSUPRESSIN OR (CARBAMOHYDROXAMIC OR CARBAMOHYDROXIMIC)(W)ACID
L3          692 S CARBAMOYL(W)(OXIME OR HYDROXAMATE) OR DROXIA OR HIDRIX OR HYDREA OR
                HYDROXYCARBAMINE)
L4          126 S CARBAMOYLHYDROXYLAMINE OR ONCO(W)CARBIDE OR OXYUREA OR LITALIR
L5            2 S AMINOCARBONYL(W)HYDROXYLAMINE
L6        53962 S L1-L5
                SAVE L6 X710NAMES/Q
L7      5323322 S TRIAL? OR (CLINICAL OR PHASE OR MULTICENTER OR MULTI(W)CENTER OR
                RANDOMIZED)(6A)(STUDY OR STUDIES)
L8         8570 S L7 AND L6
L9           47 S L6 AND (HOSPITAL OR INSTITUTION?)(3A)EXPERIENCE?
L10        8590 S L8 OR L9
L11        2245 S L10 AND (2005-2010)/PY
                SET DUPORDER FILE
L12        1620 DUP REM L11 (625 DUPLICATES REMOVED)
            189 ANSWERS '1-189' FROM FILE MEDLINE
              1 ANSWER '190' FROM FILE CABA
              6 ANSWERS '191-196' FROM FILE IPA
            172 ANSWERS '197-368' FROM FILE BIOSIS
             29 ANSWERS '369-397' FROM FILE TOXCENTER
           1185 ANSWERS '398-1582' FROM FILE EMBASE
             32 ANSWERS '1583-1614' FROM FILE ESBIOBASE
              6 ANSWERS '1615-1620' FROM FILE PASCAL
L13        1620 SORT L12 1-1620 TI
                SAVE L13 X710TRIALS/A
L14        9923 S L6 AND (2005-2010)/PY
L15        7678 S L14 NOT L11
L16         629 S L15 AND (REPRODUCT? OR DEVELOPMENTAL? OR TERATO? OR DEFORMIT
L17         128 S L15 AND ((FOETAL OR FETAL) NOT (FOETAL OR FETAL)(W)(HEMOGLOB
L18         340 S L15 AND (EMBRYO? OR GERM(W)CELL? OR TESTIS OR TESTES OR TEST
L19         324 S L15 AND (BEHAVIOR? OR BEHAVIOUR? OR MALFORM? OR PREGNAN? OR
L20         334 S L15 AND (VIABILITY OR FERTIL? OR RESORB? OR RESORP? OR STILL
L21         274 S L15 AND (UTERO OR UTERUS OR UTERINE OR MATERNAL? OR FSH OR H
L22         111 S L15 AND (GROWTH(6A)(RETARD? OR EFFECT? OR INFLUENC? OR STUNT
L23        1049 S L15 AND (PHARMACOKINETIC? OR AZOOSPERMIA OR RISK? OR MEIO? O
L24          99 S L15 AND (SPERM? OR SEMEN OR SEMINIFEROUS OR NEUROTERATO? OR
L25         140 S L15 AND (BIRTH OR SKELETAL OR NEURAL(W)TUBE(W)DEFECT? OR GER
L26        2439 S L16-L25
L27        1432 DUP REM L26 (1007 DUPLICATES REMOVED)
            341 ANSWERS '1-341' FROM FILE MEDLINE
             26 ANSWERS '342-367' FROM FILE CABA
             12 ANSWERS '368-379' FROM FILE IPA
```

```
             265 ANSWERS '380-644' FROM FILE BIOSIS
             187 ANSWERS '645-831' FROM FILE TOXCENTER
             485 ANSWERS '832-1316' FROM FILE EMBASE
             106 ANSWERS '1317-1422' FROM FILE ESBIOBASE
              10 ANSWERS '1423-1432' FROM FILE PASCAL
L28         1432 SORT L27 1-1432 TI
                 SAVE L28 X710TERAT/A
```

March 22, 2010

```
L29          357 S L15 AND (PRENATAL? OR PERINATAL? OR POSTNATAL? OR
                 DIFFERENTIATION OR GESTAT? OR ORGANOGENE?)
L30          131 S L29 NOT L26
                 SET DUPORDER FILE
L31           77 DUP REM L30 (54 DUPLICATES REMOVED)
              24 ANSWERS '1-24' FROM FILE MEDLINE
               2 ANSWERS '25-26' FROM FILE CABA
               1 ANSWER '27' FROM FILE IPA
               6 ANSWERS '28-33' FROM FILE BIOSIS
               8 ANSWERS '34-41' FROM FILE TOXCENTER
              26 ANSWERS '42-67' FROM FILE EMBASE
              10 ANSWERS '68-77' FROM FILE ESBIOBASE
L32           77 SORT L31 1-77 TI
                 SAVE L45 X710MOTERAT/A
L33         5126 L15 NOT (L26 OR L30)
L34         2965 DUP REM L33 (2161 DUPLICATES REMOVED)
             845 ANSWERS '1-845' FROM FILE MEDLINE
              13 ANSWERS '846-858' FROM FILE AGRICOLA
              11 ANSWERS '859-869' FROM FILE CABA
              18 ANSWERS '870-887' FROM FILE IPA
             402 ANSWERS '888-1289' FROM FILE BIOSIS
             651 ANSWERS '1290-1940' FROM FILE TOXCENTER
             926 ANSWERS '1941-2866' FROM FILE EMBASE
              77 ANSWERS '2867-2943' FROM FILE ESBIOBASE
              22 ANSWERS '2944-2965' FROM FILE PASCAL
L35         2433 L34 NOT PATENT/DT
L36         2433 DUP REM L35 (0 DUPLICATES REMOVED)
             845 ANSWERS '1-845' FROM FILE MEDLINE
              13 ANSWERS '846-858' FROM FILE AGRICOLA
              11 ANSWERS '859-869' FROM FILE CABA
              18 ANSWERS '870-887' FROM FILE IPA
             400 ANSWERS '888-1287' FROM FILE BIOSIS
             121 ANSWERS '1288-1408' FROM FILE TOXCENTER
             926 ANSWERS '1409-2334' FROM FILE EMBASE
              77 ANSWERS '2335-2411' FROM FILE ESBIOBASE
              22 ANSWERS '2412-2433' FROM FILE PASCAL
L37         2433 SORT L49 1-2433 TI
                 SAVE L37 X710REST/A
```

April 25 and November 15, 2011 Updates

An updated search was conducted on April 25 and November 15, 2011, using only PubMed to identify potential literature. Search terms included "hydroxyurea," "hydroxycarbamide," and "hydroxyl AND urea." The searchs were limited to articles published from February 2010 to the April 2011 and from April 2011 to November 2011, respectively. A total of 370 articles were identified in the April 25th search and 205 articles were identified in the November 15th search. From the April 25th search, 81 abstracts were reviwed further and 38 full articles were retrieved. From the November 15th search, these numbers were 53 and 42, respectively.

Additionally, articles were searched for authors identified as principal investigators of clinical trials that were focused on assessing long-term effects of hydroxyurea in children to assess whether results from these studies had been published. For those authors where greater than 20 potential articles were identified, searches were limited using keywords including "sickle cell," "hydroxyurea," "meningioma," and "desmoid." All articles not retrieved in the above search were then obtained for review.

Appendix C: MetaDrug™ Analysis Report - Hydroxyurea

Background

The summary provides an overview of the MetaDrug™ analysis method and the results of the quantitative-structure activity relationship (QSAR) analysis conducted on HU. The background information provided in this summary was obtained from the GeneGo Help Section (2011a), unless otherwise noted.

Overview of MetaDrug Analysis Methodology

MetaDrug, from GeneGo, Inc., combines chemical structural analysis tools (metabolite prediction, QSAR, structural similarity searching), a structure-activity database, and a systems biology database of molecular interactions (protein-protein, compound-protein, protein-enzymatic reaction, compound-enzymatic reaction), canonical signaling and metabolic pathways, and gene-biological property associations.

The MetaDrug analysis starts with uploading a chemical structure. Potential metabolites for the query compound are predicted and separated into major and minor phase 1 and phase 2 metabolites. A suite of pre-defined QSAR models is used to predict chemical and biological properties of the molecule (and, optionally, its metabolites). These include models for substrate affinity, inhibition of metabolic enzymes and transporters, water solubility, blood-brain barrier penetration, and plasma protein binding.

MetaDrug uses three methods with which to associate compounds to protein targets, which are subsequently subjected to functional analysis. The first method uses MetaBase database, which contains compound-protein interactions. This database directly allows compounds with known biological activities to be incorporated into networks and their pharmacological properties further investigated. The second method uses QSAR predictions of protein target affinity from the included models that define a limited number of potential targets for novel molecules and/or their metabolites submitted for analysis. The third method performs a similarity search for the structure and its major metabolites against the database of existing structures and their targets. Potential targets for novel molecules are inferred through structurally similar compounds in the database (GeneGo, personal communication).

Having defined a list of known and predicted targets using the above approach, MetaDrug uses enrichment analysis (hypergeometric distribution) of the list across nine pre-defined biological ontologies to identify biological pathways, biological, metabolic, or toxicological processes, or diseases that may be affected by interaction of the query compound and its metabolites with biological systems. These are reported as enrichment scores (-log of the hypergeometric p-value) for the top 11 enriched categories in each ontology and, for canonical pathway maps, images of the top three enriched pathway maps with predicted targets of the query compound flagged (GeneGo, personal communication).

Metabolites

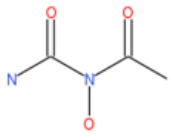
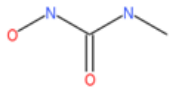
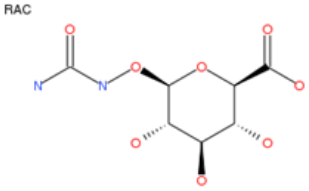
MetaDrug predicts first-pass and second-pass metabolites. Reactions are classified as Phase 1 and Phase 2, respectively. Phase 1 metabolic reactions typically include non-synthetic reactions (e.g., oxidation, reduction, and hydrolysis). These reactions are typically catalyzed by cytochrome P450 (CYP450) enzymes to increase chemical solubility. Phase 2 reactions include

conjugation reactions with glucuronic acid, sulfate, glutathione, and amino acids. These reactions are proposed to target the chemical for excretion. Seventy-four metabolic pathways (49 Phase 1 and 25 Phase 2) are used to predict metabolites. [Note: The help section notes that there are 66 metabolic rules; however, the total number of rules noted in the help section is 74.] The metabolic pathways describe "most likely metabolic reactions categorized according to the particular type of chemical transformation (e.g., aromatic hydroxylation or ester hydrolysis)." Phase 1 pathways include: C-oxidation, quinone formation, N-oxidation, S-oxidation, P-oxidation, spontaneous (e.g., ketone tautomerization, vicdiol to aldehyde), reduction, and hydrolysis. Phase 2 pathways include: glucuronide transfer, sulfate transfer, glutathione transfer, methyl transfer, cysteine transfer, other conjugation reactions (e.g., O-phosphate transfer), conjugation of amino acids, and N-acetyl transfer.

The metabolic pathways were derived from the analysis of a manually annotated human drug metabolism database that includes xenobiotic reactions, enzyme substrates, and enzyme inhibitors with kinetic data. MetaDrug also includes rules to predict and identify likely reactive metabolites (e.g., quinines and phenols).

In addition to classification as first-pass or second-pass metabolites, metabolites are further classified as predicted major or minor metabolites. The classification of major and minor metabolites is based on a score identified as the occurrence rate (OC). The OC is the "ratio of the occurrence of a particular metabolic reaction to the total number of metabolic reactions in the MetaCore™/MetaDrug™ database." The occurrence frequency is assigned to a metabolite as the negative log value. The greater the score, the higher the frequency the predicted metabolic reaction is present in the database. Major predicted metabolites have the highest OC values. Predicted metabolites are also identified as major metabolites "if they are produced by specific metabolic reactions, or when unique or highly reactive substructures undergo a transformation."

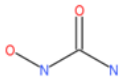
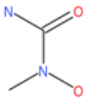
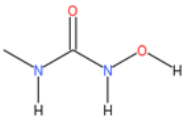
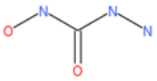
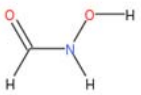
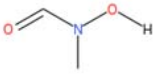
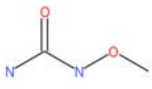
Below are the first-pass major and minor metabolites that are predicted to occur with HU.

Metabolites					
Pass	**Name**	**Structure**	**SMILES**	**Formula**	**MW**
First pass Conjugated metabolites	Hydroxycarbamide_N-acetyl_transfer1		CC(=O)N(C(=O)N)O	C3H6N2O3	118.04
First pass Conjugated metabolites	Hydroxycarbamide_N-methyl_transfer1		CNC(=O)NO	C2H6N2O2	90.04
First pass Conjugated metabolites	Hydroxycarbamide_O-glucuronide_transfer1		O=C(O)C1OC(C(O)C(O)C1O)ONC(=O)N	C7H12N2O8	252.06

Structurally Similar Chemicals in Database and QSAR Models

Structural Similarity

Based on the hypothesis that structurally similar compounds produce similar biological effects, similarity searches are conducted by searching the MetaCore™/MetaDrug™ database and results are ranked based on similarity (%). Two-dimensional fingerprints are developed for each chemical using the Accelrys Accord Cartridge. "Fingerprints are arrays generated for each molecule and containing as its elements binary hashes representing particular substructures (patterns) within that molecule." Similarity is quantified with the Tanimoto coefficient. The Tanimoto coefficient ranges from 0 to 1 and represents the ratio of the number of common fragments to the total number of fragments for two molecules. The greater the value, the greater degree of similarity noted.

Similar compounds for input molecule						
#	Compoun	Structure	Drug	Input mol	Similarity	Network
1	Hydroxycarbamide		drug	Hydroxycarbamide	100	Yes
2	1-hydroxy-1-methyl-urea			Hydroxycarbamide	80	Yes
3	3-hydroxy-1-methyl-urea			Hydroxycarbamide	80	
4	1-amino-3-hydroxy-urea			Hydroxycarbamide	75	
5	N-hydroxyformamide			Hydroxycarbamide	75	
6	N-Hydroxy-N-methyl-formamide			Hydroxycarbamide	71.43	
7	methoxyurea			Hydroxycarbamide	70.59	

QSAR

MetaDrug uses the ChemTree™ (Golden Helix) software with recursive partitioning algorithm to calculate QSAR models. A suite of pre-defined QSAR models is used to predict chemical and

biological properties of the molecule (and, optionally, its metabolites) such as absorption, metabolism, distribution, excretion, and toxicology. Each model is developed based on literature and/or manually annotated training sets from MetaCore™/MetaDrug™ database.

The recursive partitioning method used in the ChemTree software separates data based on relationships between independent (e.g., atom connectivity) and dependent (e.g., activity) variables. Data separation continues (into nodes) until no further partitions can be made based on pre-defined stopping rules. Parameters that may be adjusted include path length (minimum number of compounds that must be present for a descriptor to be included), maximum segments (maximum number of nodes for any data separation), p-value threshold (disallows any split where the p-value is greater than the threshold), and number of random trees (maximum number of trees that can be generated).

Predicted activity is classified as active or non-active based on calculated values. For non-binary QSAR algorithms, values must comply with two QSAR thresholds to be classified as active. One threshold corresponds to the negative logarithm of activity value of the most active compound of the training set, which defines the predictability limit of the model. The second threshold is the negative logarithm of 50 μM (-1.7), which is considered the lower limit for active chemicals. If the QSAR value falls within these two thresholds, the compound is considered active. For binary QSAR models, values range from 0 to 1.

For non-binary QSAR models, the ideal training set would contain data as similar as possible (e.g., from the same origin, cell line, and experiment type). For the best results in developing binary QSAR models, the training sets used contained approximately equal numbers of positives and negatives. Examples of positives for therapeutic effects included marketed drugs, drug candidates in clinical trials, and preclinical compounds with in vivo activity. Chemicals that produce specific adverse effects were defined as producing toxic effects. Chemicals present in the database that produced a particular effect were assigned an arbitrary value of 1, while those that did not produce those effects were assigned a value of 0.

A percentage, representing the Tanimoto (structural) similarity to the most similar structure in the model's training set, is displayed in parentheses below the model. Results are color coded green or red. For pharmacological models, green color indicates an activity passing the cutoff threshold (thresholds are user adjustable; this report uses the default values given in the model description). For binary models, a probability >0.5 is colored green for target-based or therapeutic models, whereas toxicity models are colored red at >0.5 probability.

QSAR modeling results indicate the following potential properties of HU (TP values >50%):

- Activity against cancer (0.90, TP = 100.00)
- Mutagen (0.60, TP = 100.00)
- Carcinogen (1; TP = 53.33)
- Carcinogen in female mice (1; TP = 53.33)
- Carcinogen in male mice (1; TP = 53.33)
- Heptatoxicant (0.73; TP = 52.94)

Possible Targets for Input Molecule

Compound-target associations are based on the premise that structurally similar compounds have similar biological function. Reported are the predicted target, the input compound (MD object), Tanimoto similarity score (%), MetaDrug database compound to which the input compound is similar, effect of MetaDrug database compound on the target, and references to the literature used to make the compound-target associations.

Possible targets for input molecule

#	Target	Type	MD object	Similarity, %	Metadrug compound	Effect	Pubmed / Patent ID
1	RRM1		Hydroxycarbamide	100	Hydroxycarbamide	inhibition	458812,926124,17324380
2	RRM1		1-hydroxy-1-methyl-urea	80	Hydroxycarbamide	inhibition	926124
3	Carbonic anhydrase II		Hydroxycarbamide	100	Hydroxycarbamide	inhibition	12713833,16759856,17251018
4	Carbonic anhydrase IV		Hydroxycarbamide	100	Hydroxycarbamide	inhibition	16759856
5	Carbonic anhydrase I		Hydroxycarbamide	100	Hydroxycarbamide	inhibition	12713833
6	Carbonic anhydrase IX		Hydroxycarbamide	100	Hydroxycarbamide	inhibition	16759856,17251018
7	Ribonucleotide reductase		Hydroxycarbamide	100	Hydroxycarbamide	inhibition	14729598,12168813,12967138
8	RRM2		Hydroxycarbamide	100	Hydroxycarbamide	inhibition	458812,926124
9	RRM2		1-hydroxy-1-methyl-urea	80	Hydroxycarbamide	inhibition	926124
10	Carbonic anhydrase VA		Hydroxycarbamide	100	Hydroxycarbamide	inhibition	16759856

Based on literature reports and structural similarity to 1-hydroxy-1-methylurea, subunits of ribonucleotide reductase and various forms of carbonic anhydrase were identified as possible targets.

GeneGo Functional Ontologies

Enrichment analysis of the identified target list is shown across nine functional biology ontologies. The enrichment calculation uses the Fisher's exact test or hypergeometric distribution to calculate the probability that the degree of overlap between the list of possible protein targets generated from the query compound analysis and the proteins represented in the functional ontology category can happen by chance given an identical number of proteins selected at random from the universe of proteins annotated within the ontology. The p-value generated is used to rank order the categories within each ontology by their significance to the list of targets, thereby identifying maps or biological processes likely to be affected by compound exposure (GeneGo, personal communication).

GeneGo Maps

Name	Map	pValue
Hydroxycarbamide	Nitrogen metabolism	5.276e-11
Hydroxycarbamide	Nitrogen metabolism/ Rodent version	6.125e-11
Hydroxycarbamide	dCTP/dUTP metabolism	3.750e-05
Hydroxycarbamide	dATP/dITP metabolism	7.860e-05
Hydroxycarbamide	ATP/ITP metabolism	1.725e-04
Hydroxycarbamide	Oxidative stress_Role of ASK1 under oxidative stress	3.206e-02
Hydroxycarbamide	Transcription_Role of VDR in regulation of genes involved in osteoporosis	5.506e-02
Hydroxycarbamide	dGTP metabolism	6.050e-02

GeneGo Process Networks		
Name	**Network**	**pValue**
Hydroxycarbamide	Transport_Iron transport	2.870e-02
Hydroxycarbamide	Signal transduction_NOTCH signaling	6.640e-02

GeneGo Disease Biomarker Networks		
Name	**Network**	**pValue**
Hydroxycarbamide	Breast neoplasm_Endothelins	2.630e-02

GeneGo Drug Target Networks		
Name	**Network**	**pValue**
Hydroxycarbamide	Metabolism_Carbonic anhydrase I	8.331e-08
Hydroxycarbamide	Signal transduction_GPCR, p53 signaling	1.029e-01

GO Processes		
Name	**Process**	**pValue**
Hydroxycarbamide	one-carbon metabolic process	3.804e-09
Hydroxycarbamide	deoxyribonucleotide biosynthetic process	1.086e-08
Hydroxycarbamide	deoxyribonucleotide metabolic process	3.250e-07
Hydroxycarbamide	deoxyribonucleoside diphosphate metabolic process	2.834e-06
Hydroxycarbamide	nucleoside diphosphate metabolic process	6.599e-05
Hydroxycarbamide	DNA replication	1.256e-04
Hydroxycarbamide	nucleotide biosynthetic process	1.588e-04
Hydroxycarbamide	protein oligomerization	2.646e-04
Hydroxycarbamide	nucleoside phosphate metabolic process	6.306e-04
Hydroxycarbamide	nucleotide metabolic process	6.306e-04
Hydroxycarbamide	response to steroid hormone stimulus	6.828e-04
Hydroxycarbamide	nucleobase, nucleoside and nucleotide metabolic process	8.281e-04
Hydroxycarbamide	cellular metabolic process	2.130e-03
Hydroxycarbamide	DNA metabolic process	2.255e-03
Hydroxycarbamide	positive regulation of cellular pH reduction	2.322e-03
Hydroxycarbamide	carbon dioxide transport	2.322e-03
Hydroxycarbamide	positive regulation of bone resorption	2.786e-03
Hydroxycarbamide	positive regulation of bone remodeling	2.786e-03
Hydroxycarbamide	regulation of cellular pH reduction	2.786e-03
Hydroxycarbamide	kidney development	2.987e-03
Hydroxycarbamide	response to hormone stimulus	3.557e-03
Hydroxycarbamide	protein complex assembly	3.828e-03
Hydroxycarbamide	protein complex biogenesis	3.828e-03
Hydroxycarbamide	mitochondrial DNA replication	4.640e-03
Hydroxycarbamide	positive regulation of osteoclast differentiation	4.640e-03
Hydroxycarbamide	oxidation reduction	4.668e-03
Hydroxycarbamide	morphogenesis of an epithelium	4.763e-03
Hydroxycarbamide	response to endogenous stimulus	4.802e-03
Hydroxycarbamide	mitochondrial DNA metabolic process	5.103e-03
Hydroxycarbamide	urogenital system development	5.257e-03
Hydroxycarbamide	metabolic process	5.867e-03
Hydroxycarbamide	positive regulation of tissue remodeling	6.029e-03
Hydroxycarbamide	deoxyribonucleoside triphosphate metabolic process	6.491e-03
Hydroxycarbamide	osteoclast differentiation	7.415e-03
Hydroxycarbamide	macromolecular complex assembly	7.707e-03
Hydroxycarbamide	mitochondrial genome maintenance	9.262e-03
Hydroxycarbamide	regulation of bone resorption	9.262e-03
Hydroxycarbamide	macromolecular complex subunit organization	9.283e-03
Hydroxycarbamide	regulation of bone remodeling	9.723e-03
Hydroxycarbamide	tissue morphogenesis	1.094e-02

Hydroxycarbamide	gas transport	1.157e-02
Hydroxycarbamide	response to zinc ion	1.157e-02
Hydroxycarbamide	response to pH	1.157e-02
Hydroxycarbamide	response to testosterone stimulus	1.249e-02
Hydroxycarbamide	regulation of cellular pH	1.340e-02
Hydroxycarbamide	regulation of tissue remodeling	1.386e-02
Hydroxycarbamide	gluconeogenesis	1.386e-02
Hydroxycarbamide	epithelium development	1.503e-02
Hydroxycarbamide	secretion	1.714e-02
Hydroxycarbamide	cellular component assembly	1.731e-02

Go Molecular Functions

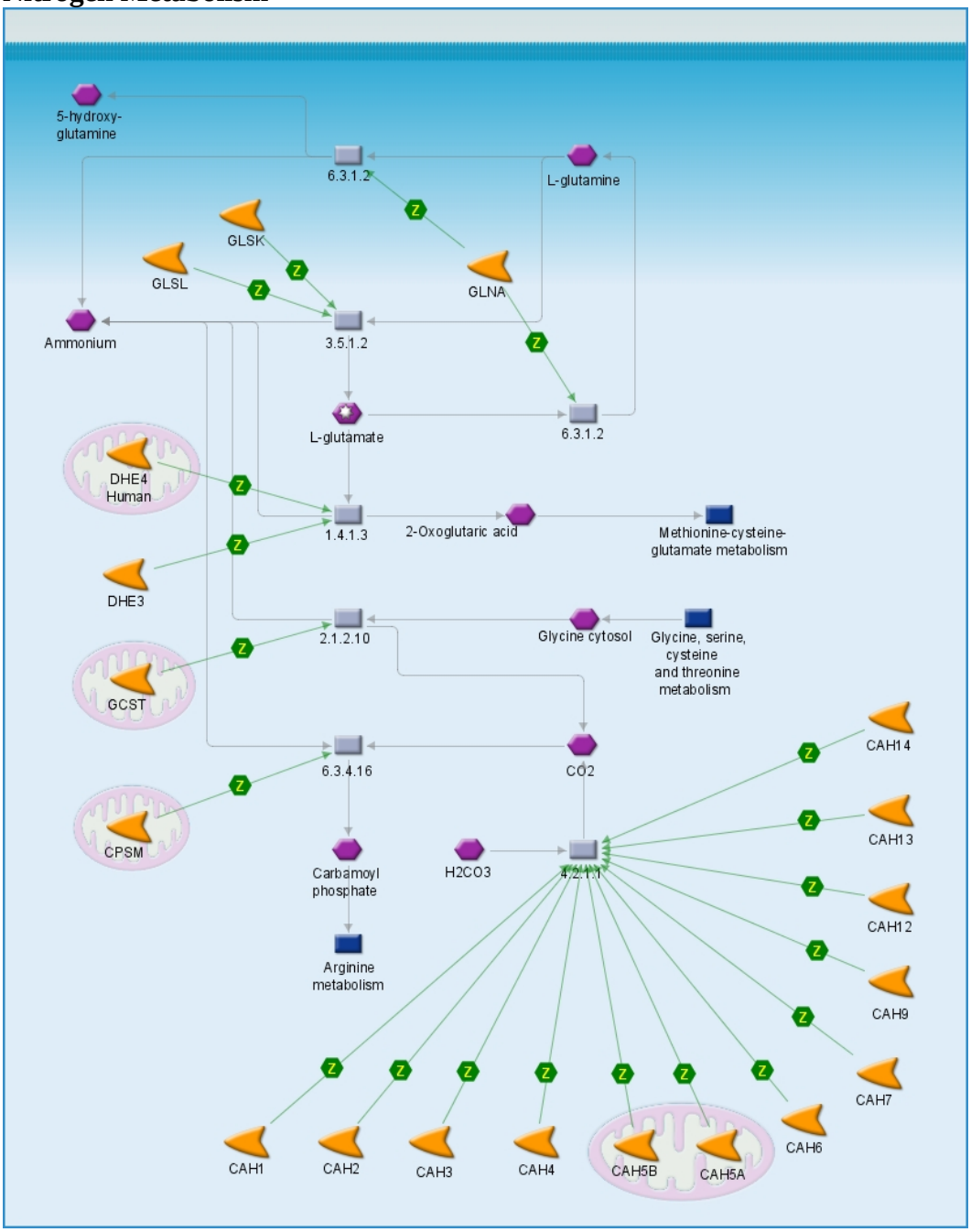
Name	Function	pValue
Hydroxycarbamide	carbonate dehydratase activity	2.359e-14
Hydroxycarbamide	hydro-lyase activity	3.411e-12
Hydroxycarbamide	carbon-oxygen lyase activity	1.003e-11
Hydroxycarbamide	lyase activity	1.175e-09
Hydroxycarbamide	ribonucleoside-diphosphate reductase activity	6.633e-07
Hydroxycarbamide	oxidoreductase activity, acting on CH or CH2 groups, disulfide as acceptc	6.633e-07
Hydroxycarbamide	oxidoreductase activity, acting on CH or CH2 groups	2.321e-06
Hydroxycarbamide	transition metal ion binding	1.270e-04
Hydroxycarbamide	catalytic activity	3.022e-04
Hydroxycarbamide	zinc ion binding	7.535e-04
Hydroxycarbamide	metal ion binding	1.225e-03
Hydroxycarbamide	cation binding	1.293e-03
Hydroxycarbamide	ion binding	1.391e-03
Hydroxycarbamide	oxidoreductase activity	3.840e-02
Hydroxycarbamide	binding	1.054e-01
Hydroxycarbamide	iron ion binding	1.459e-01
Hydroxycarbamide	ATP binding	4.794e-01
Hydroxycarbamide	adenyl ribonucleotide binding	4.855e-01
Hydroxycarbamide	adenyl nucleotide binding	5.051e-01
Hydroxycarbamide	purine nucleoside binding	5.133e-01
Hydroxycarbamide	nucleoside binding	5.155e-01
Hydroxycarbamide	purine ribonucleotide binding	5.580e-01
Hydroxycarbamide	ribonucleotide binding	5.582e-01
Hydroxycarbamide	purine nucleotide binding	5.755e-01
Hydroxycarbamide	nucleotide binding	6.362e-01
Hydroxycarbamide	protein binding	7.270e-01

GO Localizations		
Name	**Localization**	**pValue**
Hydroxycarbamide	ribonucleoside-diphosphate reductase complex	4.196e-04
Hydroxycarbamide	apical part of cell	3.544e-03
Hydroxycarbamide	basolateral plasma membrane	4.100e-03
Hydroxycarbamide	sarcoplasmic reticulum	1.873e-02
Hydroxycarbamide	microvillus	1.997e-02
Hydroxycarbamide	sarcoplasm	1.997e-02
Hydroxycarbamide	cytosol	2.207e-02
Hydroxycarbamide	sarcolemma	3.512e-02
Hydroxycarbamide	cell projection	5.695e-02
Hydroxycarbamide	apical plasma membrane	6.797e-02
Hydroxycarbamide	cytoplasmic part	6.831e-02
Hydroxycarbamide	cytoplasm	7.030e-02
Hydroxycarbamide	intracellular part	7.557e-02
Hydroxycarbamide	plasma membrane part	8.285e-02
Hydroxycarbamide	axon	8.945e-02
Hydroxycarbamide	intracellular	9.006e-02
Hydroxycarbamide	anchored to membrane	1.002e-01
Hydroxycarbamide	mitochondrial matrix	1.048e-01
Hydroxycarbamide	mitochondrial lumen	1.048e-01
Hydroxycarbamide	intracellular membrane-bounded organelle	1.440e-01
Hydroxycarbamide	membrane-bounded organelle	1.446e-01
Hydroxycarbamide	subsynaptic reticulum	1.782e-01
Hydroxycarbamide	neuron projection	1.887e-01
Hydroxycarbamide	intracellular organelle lumen	2.201e-01
Hydroxycarbamide	nucleus	2.225e-01
Hydroxycarbamide	organelle lumen	2.276e-01
Hydroxycarbamide	intracellular organelle	2.310e-01
Hydroxycarbamide	organelle	2.334e-01
Hydroxycarbamide	membrane-enclosed lumen	2.349e-01
Hydroxycarbamide	mitochondrial part	2.619e-01
Hydroxycarbamide	nucleolus	2.953e-01
Hydroxycarbamide	plasma membrane	3.048e-01
Hydroxycarbamide	membrane fraction	3.845e-01
Hydroxycarbamide	insoluble fraction	3.967e-01
Hydroxycarbamide	endoplasmic reticulum	4.042e-01
Hydroxycarbamide	extracellular space	4.324e-01
Hydroxycarbamide	cell fraction	4.818e-01
Hydroxycarbamide	extracellular region part	4.905e-01
Hydroxycarbamide	mitochondrion	4.979e-01
Hydroxycarbamide	nuclear lumen	5.322e-01
Hydroxycarbamide	nuclear part	6.193e-01
Hydroxycarbamide	cell part	6.194e-01
Hydroxycarbamide	cell	6.197e-01
Hydroxycarbamide	membrane part	6.494e-01
Hydroxycarbamide	intracellular organelle part	6.547e-01
Hydroxycarbamide	organelle part	6.591e-01
Hydroxycarbamide	extracellular region	7.038e-01
Hydroxycarbamide	intracellular non-membrane-bounded organelle	7.542e-01
Hydroxycarbamide	non-membrane-bounded organelle	7.542e-01
Hydroxycarbamide	protein complex	7.595e-01

Top GeneGo Pathway Maps

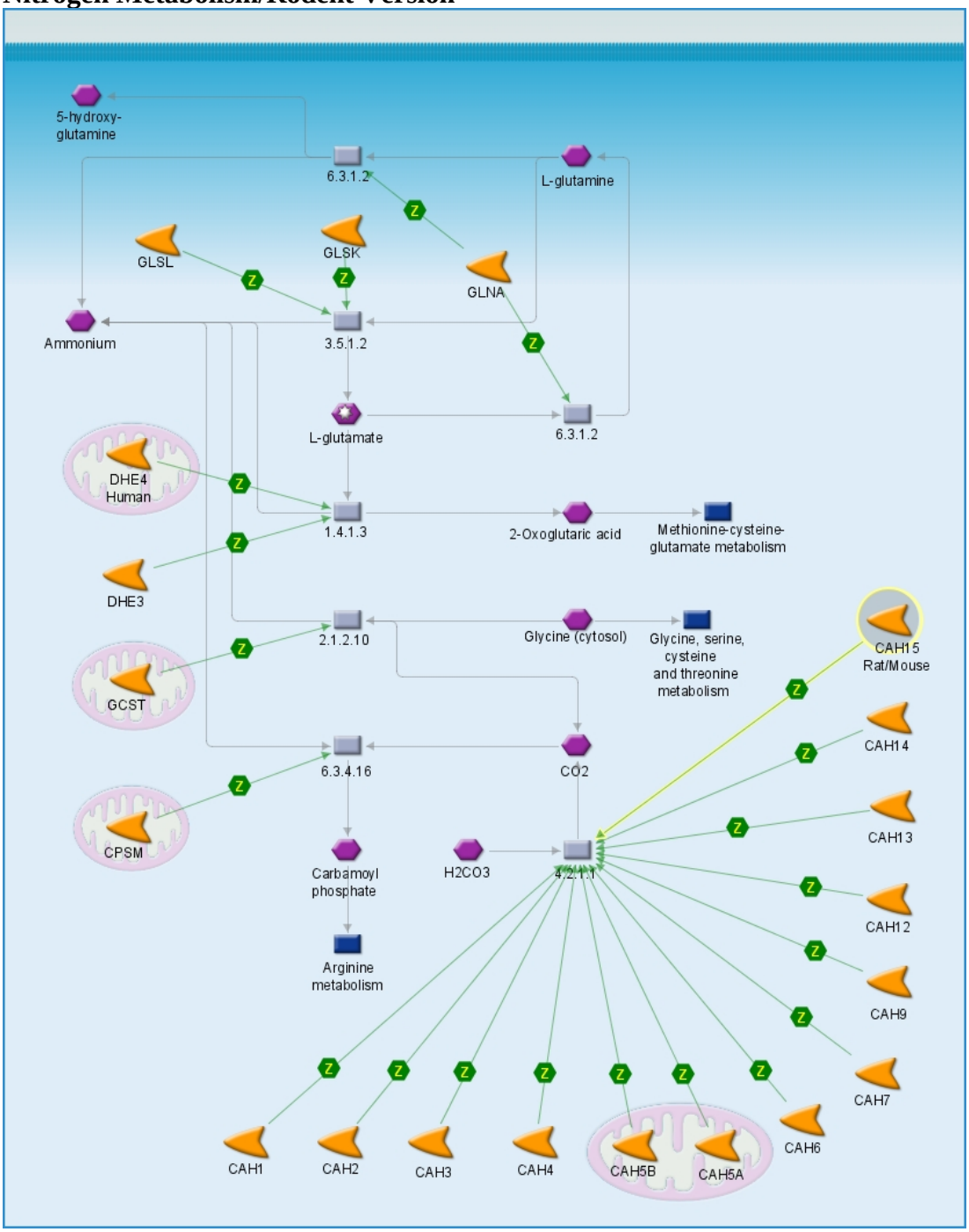
GeneGo pathway maps comprise pictorial representations of human and rodent signaling and metabolic pathways. The three most significant maps are shown below. Compounds are represented by purple hexagons, proteins by colored shapes representing different classes of compound, and enzymatic reactions by gray rectangles. Protein-protein, compound-protein, and compound-reaction interactions are shown as unidirectional arrows, and a mechanism of interaction is represented by letters in hexagonal boxes over the arrows.

Nitrogen Metabolism



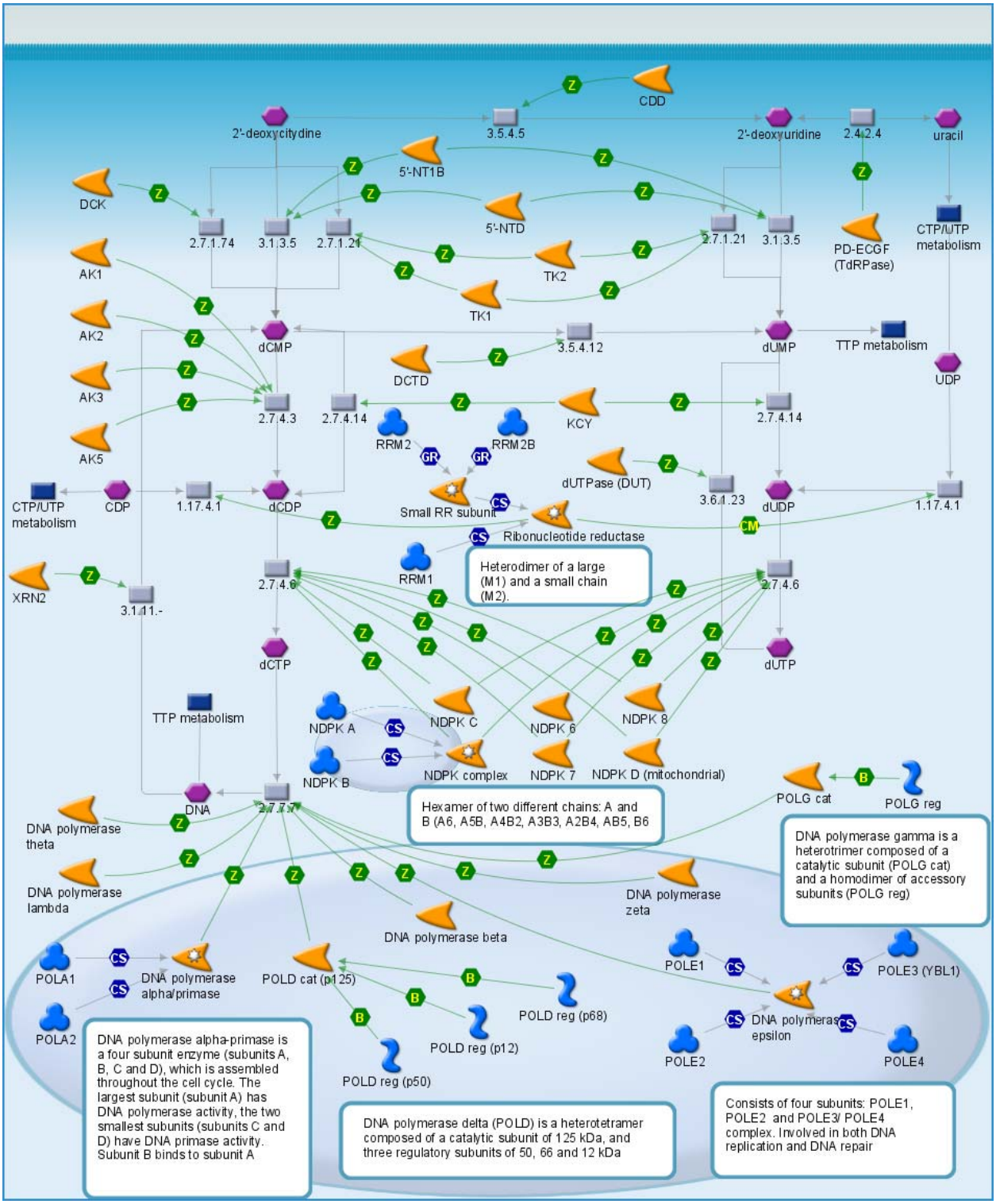
Nitrogen metabolism is a necessary metabolic process that occurs in organisms. In higher organisms nitrogen, in the form of ammonium, can either be converted to urea and excreted in urine or used in amination reactions during the *de novo* synthesis of amino acids. The latter process occurs through conjugation of ammonium with carbon dioxide (CO^{-2}) to produce carbamoyl phosphate, which then is involved in arginine metabolism. The CO^{-2} used in this reaction is produced by decomposition of bicarbonate by a variety of carbonic anhydrases, including carbonic anhydrase I, II. IV. VA. and IX (GeneGo, 2011b).

Nitrogen Metabolism/Rodent Version



As stated above, nitrogen metabolism is a necessary metabolic process that occurs in organisms. Nitrogen, in the form of ammonium, can be used in amination reactions during the *de novo* synthesis of amino acids. This process occurs through conjugation of ammonium with carbon dioxide (CO^{-2}) to produce carbamoyl phosphate, which then is involved in arginine metabolism. The CO-2 used in this reaction is produced by decomposition of bicarbonate by a variety of carbonic anhydrases, including carbonic anhydrase I, II. IV. VA. and IX (GeneGo, 2011b).

dCTP/dUTP Metabolism



Deoxyribonucleotides are needed for DNA repair and synthesis. Ribonucelotide reductase plays an essential role in formation of deoxyribonucleotides by catalyzing the reduction of ribonucleotides. Inhibition of ribonucleotide reductase leads to decreased cellular levels of deoxyribonucleotides (Sigmond et al., 2007).

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